A COMPARATIVE EVALUATION OF SERUM HEPCIDIN AND INTERLEUKIN-6 LEVELS BEFORE AND AFTER PHASE I PERIODONTAL THERAPY IN PATIENTS WITH CHRONIC AND AGGRESSIVE PERIODONTITIS

A Dissertation submitted in
partial fulfillment of the requirements
for the degree of

MASTER OF DENTAL SURGERY

BRANCH – II
PERIODONTOLOGY

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
Chennai – 600 032

2016 - 2019

CERTIFICATE BY THE GUIDE

This is to certify that **Dr.S.AISWARYA**, Post Graduate student (2016–2019) in the Department of Periodontology, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003 has done this dissertation titled **"A COMPARATIVE EVALUATION OF SERUM HEPCIDIN AND INTERLEUKIN-6 LEVELS BEFORE AND AFTER PHASE I PERIODONTAL THERAPY IN PATIENTS WITH CHRONIC AND AGGRESSIVE PERIODONTITIS"** under my direct guidance and supervision in partial fulfilment of the regulations laid down by **Tamil Nadu Dr. M.G.R. Medical University**, Chennai – 600 032 for **M.D.S.,** (Branch – II) **Periodontology** degree examination.

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DECLARATION BY THE CANDIDATE

TITLE OF STUDY	A COMPARATIVE EVALUATION OF SERUM HEPCIDIN AND INTERLEUKIN-6 LEVELS BEFORE AND AFTER PHASE I PERIODONTAL THERAPY IN PATIENTS WITH CHRONIC AND AGGRESSIVE PERIODONTITIS
PLACE OF STUDY	TAMIL NADU GOVERNMENT DENTAL COLLEGE AND HOSPITAL, CHENNAI – 600 003.
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I hereby declare that this dissertation titled **"A COMPARATIVE EVALUATION OF SERUM HEPCIDIN AND INTERLEUKIN-6 LEVELS BEFORE AND AFTER PHASE I PERIODONTAL THERAPY IN PATIENTS WITH CHRONIC AND AGGRESSIVE PERIODONTITIS"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.BHUVANESWARI.P, M.D.S., Professor and Guide,** Department of Periodontology, Tamil Nadu Government Dental College and Hospital, Chennai - 600003.

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Last, but not the least, I thank **GOD ALMIGHTY** for his blessings.

TRIPARTITE AGREEMENT

This agreement herein after the "Agreement" is entered into on this day -------- between the Tamil Nadu Government Dental College and Hospital represented by its **Principal** having address at Tamil Nadu Government Dental College and Hospital, Chennai – 600 003, (hereafter referred to as, 'the college')

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Dr. S.AISWARYA, aged 26 years currently studying as **Post Graduate student** in Department of Periodontology, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003 (herein after referred to as the 'PG student and Principal Investigator'),

And

Mrs. Dr. BHUVANESWARI.P, M.D.S., aged 50 years working as **Professor** in Department of Periodontology at the Tamil Nadu Government Dental College and Hospital, Chennai – 600 003 (hereafter referred to as 'Co-investigator'),

Whereas the PG student as part of her curriculum undertakes this research on **"A COMPARATIVE EVALUATION OF SERUM HEPCIDIN AND INTERLEUKIN-6 LEVELS BEFORE AND AFTER PHASE I PERIODONTAL THERAPY IN PATIENTS WITH CHRONIC AND AGGRESSIVE PERIODONTITIS"** for which purpose the Co – investigator and the college shall provide the requisite infrastructure based on availability and also provide facility to the PG student as to the extent possible as a Principal Investigator

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Ref No: R. C. NO: 0420/DE/2017 date : 15.05.2017

Sub: IEC review of the research proposals,

Title of the work:	A COMPARATIVE EVALUATION OF SERUM HEPCIDIN AND INTERLEUKIN–6 LEVELS BEFORE AND AFTER PHASE I PERIODONTAL THERAPY IN PATIENTS WITH CHRONIC AND AGGRESSIVE PERIODONTITIS
Investigator:	DR.S.AISWARYA, II YEAR MDS.
Department :	DEPARTMENT OF PERIODONTICS, TAMIL NADU GOVT. DENTAL COLLEGE & HOSPITAL,CHENNAI-3.

Thank you for submitting your research proposal , which was considered at the Institutional Ethics Committee meeting held on the 27/02/2017, at TN Govt. Dental College. The documents related to the study referred above were discussed and reviewed. The decision of the members of the committee , the secretary and the Chairperson IEC of TN Govt. Dental College is here under:

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CERTIFICATE

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ABSTRACT

BACKGROUND: Periodontitis causes persistent inflammation, connective tissue breakdown and alveolar bone destruction, mediated by various types of Pro-inflammatory mediators. One of such pro-inflammatory mediator is interleukin–6, which is a signalling protein/cytokine produced by several cells including macrophages, neutrophils, keratinocytes, and fibroblasts, in response to stimulus such as infection and trauma. Interleukin-6 and lipopolysaccharides from microorganisms stimulate the hepatic expression of an acute-phase protein called hepcidin, which inhibits duodenal absorption of iron. This in turn may lead to anaemia of chronic disease. In an anaemic state there is increased periodontal destruction because of relative decrease in oxygen perfusion into tissues.

AIM: The aim of this study is to determine and compare the level of serum hepcidin and interleukin-6 before and after phase-I periodontal therapy in patients with chronic and aggressive periodontitis.

MATERIALS AND METHOD: In the present study, 45 subjects were selected, of which 15 were categorized as Group I which consisted of healthy subjects, 15 were categorized as Group II which consisted of patients with chronic periodontitis and another 15 were categorized as Group III which consisted of patients with aggressive periodontitis. The clinical parameters assessed were plaque index, gingival bleeding index, pocket probing depth, clinical attachment level. The laboratory parameters assessed were serum IL-6 and hepcidin level. All the parameters were assessed at baseline in group I, at baseline and 3 months after phase I therapy in group II and III.

RESULTS: The levels of serum Hepcidin and IL-6 were elevated in chronic and aggressive periodontitis patients when compared with healthy subjects at baseline. However, there was no statistically significant difference in baseline values of Hepcidin and IL-6 between chronic and aggressive periodontitis patients. Following Phase I therapy there was a statistically significant reduction from baseline values in PI,GBI,PPD,CAL and serum levels of Hepcidin,IL-6 in chronic and aggressive periodontitis patients.

CONCLUSION: Within the limitations of this study, it could be concluded that increased serum IL-6 and hepcidin level is associated with both chronic and aggressive periodontitis patients which increases the risk for the development of anemia of chronic disease. However, there was no statistically significant difference in baseline values of Hepcidin and IL-6 between chronic and aggressive periodontitis patients. Following phase I therapy the levels of both IL-6 and Hepcidin reduced in chronic and aggressive periodontitis patients. In future, studies with a larger sample size may be employed to further establish the association between serum Hepcidin levels and periodontal status of the patient and to evaluate the efficiency of phase I therapy in reducing serum IL-6 and Hepcidin levels. This will help to reduce the systemic burden of the patient like development of anaemia, CVS complications etc.,

KEY WORDS: Hepcidin, Interleukin-6, Anaemia of chronic disease, chronic periodontitis, Aggressive periodontitis

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LIST OF ABBREVIATIONS

Abbreviation	*Expansion*
IL-6	Interleukin-6
CRP	C-reactive protein
SAA	Serum Amyloid A
AI	Anaemia of Inflammation
APP	Acute Phase Protein
TNF	Tumour necrosis factor
TGF	Transforming growth factor
IFN	Interferon
CSF	Colony stimulating factor
BSF	B-cell–stimulating factor
CAL	Clinical attachment level
TLR's	Toll like receptors
CEJ	Cemento enamel junction
PAMP's	pathogen-associated molecular patterns
NF-kB	Nuclear factor kappa B
SOCS	Suppressors of cytokine signalling
PPC	Periodontal Profile Class
CHD	Coronary heart disease
ACD	Anaemia of chronic disease
MCV	Mean corpuscular volume
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
ESR	Erythrocyte sedimentation rate

MMP's	Matrix metallo proteinases
PGE2	Prostaglandin E 2
SRP	Scaling and Root planning
TIMP's	Tissue inhibitor of matrix metalloproteinases
Hb	Haemoglobin
hsCRPs	high-sensitivity C-reactive proteins
DM	Diabetes mellitus
PCV	Packed Cell Volume
ACPA	Anti-Citrullinated protein Antibodies
LDL	Low density lipoprotein
VLDL	Very Low density lipoprotein
PI	Plaque index
PPD	Probing pocket depth
GBI	Gingival bleeding index
NSAID's	Non-steroidal anti-inflammatory drugs
OHI	Oral hygiene instructions
RBC	Red blood cells
ELISA	Enzyme linked immunosorbent assay
GCF	Gingival crevicular fluid
SD	Standard deviation
SPSS	Statistical Package for Social Science
HEPC	Hepcidin
OD	Optical density
RPM	Rotations per minute
ANOVA	Analysis of variance

INTRODUCTION

Chronic periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both **(Carranza's clinical periodontology 11th edition)**[1]

Aggressive periodontitis is the disease of the periodontium occurring in an otherwise healthy individual which is characterised by a rapid loss of alveolar bone about more than one tooth of the permanent dentition. The amount of destruction manifested is not in proportion with the amount of local irritants **(Baer et al, 1971)**

Although the presence of periodontopathogens is needed for disease initiation in both conditions, it is inadequate for progression and increased severity of disease. The onset, progression, and severity of periodontal disease are related to the interaction between periodontal microorganisms and the host immune response[2,3,4]. In response to bacterial endotoxins and mediators that cause tissue breakdown, acute phase proteins, cytokines, and prostaglandins, are produced as part of the host response.[5,6]

Acute-phase response occurs in the innate host response to injuries, infections, or ischemic necrosis by releasing various acute-phase proteins, such as C reactive protein and fibrinogen[7,8]. Data show that levels of various acute-phase proteins increased in periodontal disease in both gingival crevicular fluid (GCF) and plasma or serum.[9]

Cytokines, which are soluble proteins, play an important role in the initiation and maintenance of inflammatory and immune responses.[10] Interleukin-6 (IL-6), a multifunctional cytokine, is the major regulator of acute-phase protein synthesis during

acute-phase response and one of the most studied inflammatory markers in periodontal disease.

IL-6 is promptly produced in an infected or a damaged lesion and provides an emergent signal to the entire body. When stimulating hepatocytes, IL-6 strongly induces a broad spectrum of acute-phase proteins such as C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen, hepcidin, haptoglobin, and antichymotrypsin, whereas it reduces albumin, cytochrome p450, fibronectin, and transferrin[11].

Elevated levels of serum pro-inflammatory cytokines and inflammatory mediators, such as IL-6 and CRP, have been shown to be associated with measures of periodontal disease. Interleukin-6 and lipopolysaccharides from microorganisms stimulate the hepatic expression of the acute-phase protein hepcidin, which inhibits duodenal absorption of iron.

Hepcidin is a cationic peptide that is rich in cysteine. It is synthesized by the liver and excreted by the kidney and its main function is homeostatic regulation of iron metabolism.[12] The target for serum hepcidin is the iron exporter ferroportin-I[13], which is found in the plasma membranes of most body cells and at high concentrations in duodenal enterocytes, macrophages and hepatocytes.

Increased circulating hepcidin, due to inflammatory stimulation such as chronic periodontitis, induces internalization and degradation of ferroportin, whose loss from cell surface prevents the exit of iron from inside the cells into the plasma, resulting in low transferrin saturation which leads to the decrease of erythropoiesis, contributing to the development of anemia of chronic disease.

The anemia of chronic disease refers to the impaired production of erythrocytes associated with chronic inflammatory states, including cancer, chronic infection, or

autoimmune diseases. Recent data indicate that anemia can also occur in the setting of severe, acute inflammation, such as critical illness, or with milder but persistent inflammatory signals that occur in obesity, aging, and kidney failure. For these reasons, the name "anemia of inflammation"(AI) may be more suitable than anemia of chronic disease.[14]

In a study by **Carvalho 2015**, the serum Hepcidin and interleukin-6 levels were found to be higher in chronic periodontitis patients when compared with healthy subjects.

Currently there are no studies regarding hepcidin levels in aggressive periodontitis patients. This study will help to estimate the hepcidin levels in aggressive periodontitis patients and compare it with hepcidin levels in chronic periodontitis patients and healthy individuals and to compare serum hepcidin and interleukin-6 levels before and after phase I periodontal therapy in patients with chronic and aggressive periodontitis.

AIM AND OBJECTIVES

AIM

The aim of this study is to determine and compare the level of serum hepcidin and interleukin-6 before and after phase-I periodontal therapy in patients with chronic and aggressive periodontitis.

OBJECTIVES

- To measure serum hepcidin and interleukin-6 levels in healthy subjects.
- To measure serum hepcidin and interleukin-6 levels in patients with chronic periodontitis.
- To measure serum hepcidin and interleukin-6 levels in patients with aggressive periodontitis.
- To compare serum hepcidin and interleukin-6 levels in individuals with healthy periodontium (control group) and chronic periodontitis(study group) and aggressive periodontitis(study group)
- To measure and compare serum hepcidin and interleukin-6 levels before and after phase I periodontal therapy in patients with chronic periodontitis.
- To measure and compare serum hepcidin and interleukin-6 levels before and after phase I periodontal therapy in patients with aggressive periodontitis.
- To compare the serum IL-6 and hepcidin levels between chronic and aggressive periodontitis patients.

REVIEW OF LITERATURE

Periodontal disease is an inflammatory disease initiated by microbial infections that lead to a host response, which results in inflammatory breakdown of tooth-supporting osseous and soft tissues. The presence of periodontopathogens alone is inadequate for progression and increased severity of disease. The onset, progression, and severity of periodontal disease are related to the interaction between periodontal microorganisms and the host immune response.

In response to bacterial endotoxins, the host releases mediators that cause tissue breakdown. This includes acutephase proteins, cytokines, and prostaglandins etc.

ACUTE PHASE PROTEIN

In response to inflammation associated cytokines, the concentration of many plasma proteins increase during inflammatory states. C-reactive protein (CRP) is the first such protein to be recognized. It was initially detected in serum obtained from patients during the acute phase of pneumococcal pneumonia, hence the term 'Acute phase protein' (APP).

Acute phase proteins are commonly defined as plasma proteins whose concentrations increase **(positive acute phase proteins)** by at least 25% during inflammatory states. In addition, it has been recognized that a number of negative acute phase proteins, whose concentrations decrease significantly under these circumstances**.** Altered production of proteins by hepatocytes is the main reason for all these changes.[15]

Inducers of acute phase gene expression

The major stimuli for induction of acute phase changes are the inflammation associated cytokines. A wide variety of cytokines, growth factors, hormones, and other mediators have also been found to influence the expression of acute phase protein genes in liver or liver derived cells in culture. These molecules contribute to the inflammatory process at inflammatory sites largely by paracrine or autocrine manner but the induction of acute phase proteins changes results from bloodborne effects upon the liver. Of these cytokines, members of the IL-6 family, TNF (Tumour Necrosis factor)-α, IL-1, TGF (Transforming growth factor)-ß, and IFN (Interferon)-ɣ have been studied elaborately.

Gauldie et al., 1987 states that IL-6 is the major stimulator of most acute phase proteins. There is evidence that acute-phase cytokines and acute-phase reactants are released and associated with tissue breakdown in periodontal disease. Acute-phase response occurs in response to injuries, infections, or ischemic necrosis by releasing various acute-phase proteins, such as C reactive protein and fibrinogen. Data show that levels of various acute-phase proteins increased in periodontal disease in both gingival crevicular fluid (GCF) and plasma or serum **(Keles et al, JOP 2014).** Therefore, acute-phase proteins can be susceptibility markers in relation to inflammatory status.

BIOMARKER

A biomarker is a substance used to indicate a biologic state and gives an objective measure to evaluate the present and future disease activity. It is defined as – A substance that is measured objectively and evaluated as an indicator of normal

biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[16]Various biological media like saliva, serum and gingival crevicular fluid are used to determine biomarkers in periodontal health and disease. A single biomarker will not able to predict periodontal disease activity and severity. So a combination of biomarkers is necessaary.[17]

Several molecules have been studied as potential biomarkers for periodontal disease including enzymes, cytokines, receptors, and other proteins. The biomarkers in gingival crevicular fluid (GCF) contains local biomarkers such as matrix metalloproteinases, acid/alkaline phosphatase etc., which can potentially provide information at the site level. While, the biomarkers in the blood, serum, or plasma contain systemic biomarkers, which can potentially, provide information at the patient level; and saliva contains both local and systemically derived markers and provides information at the patient level as well.

Although the GCF has the advantage that it provides information at the site level, GCF collection is rather complicated and thus not convenient during routine dental procedures. Comparatively Saliva sample collection is easy, fast, non-invasive, and more convenient for the patient and the clinician. Saliva contains local and systemically derived markers of periodontal disease.[18]The blood cannot provide site-specific information **(Panagiota.G, Curr Oral Health Rep, 2015).** Its sampling is generally more invasive than that of saliva. But it is relatively easy, fast, and can be performed outside of the dental office and as part of a routine general diagnostic check-up. Because of the simple, fast, and relatively non-invasive method of collection, salivary and blood diagnostic tests have the potential to be used for diagnosis and monitoring of periodontal disease at the patient level.[19]

INTERLEUKIN-6

Cytokines are soluble mediators that aid cell-to-cell communication in immune responses. They include IFNs, chemokines, lymphokines, interleukins, TGF-b, colony-stimulating factors (CSF), and TNF which are characterized by functional redundancy and pleiotropy. Interleukins are cytokines that act primarily on leukocytes. Till date, nearly 40 interleukins have been identified. Interleukin-6 (IL-6) is a prototypical cytokine and an inflammatory biomarker.

At first, IL-6 was identified as B-cell–stimulating factor 2 (BSF-2) in the culture supernatants of mitogen- or antigen stimulated peripheral blood mononuclear cells. It induce immunoglobulin production in Epstein–Barr virus–transformed B-cell lines or in Staphylococcus aureus Cowan 1–stimulated B cells.[20]In 1986 the gene encoding BSF-2 was cloned.[21]

BSF-2 was found to be alike the hepatocyte-stimulating factor, the hybridoma growth factor, and IFNb-2. The molecule later became known as IL-6. Human IL-6 consists of 184 amino acids with two potential N-glycosylation sites and four cysteine residues. The core protein is about 20 kDa, and glycosylation accounts for the 21- to 26-kDa size of natural IL-6**(Hirano et al 1986).**

Biosynthesis and release

IL-6 is produced by monocytes and macrophages after the stimulation of Toll-like receptors (TLRs) with distinct pathogen-associated molecular patterns (PAMP's) during infectious inflammatory state. While, in non-infectious inflammations, such as burns or traumatic injuries, damage associated molecular patterns from damaged or

dying cells stimulate TLRs to produce IL-6. In addition to these cells, IL-6 can be produced by a variety of cells **(Akira et al. 1993)**.[22]:

1. Dendritic cells,
2. T and B cells,
3. Neutrophils,
4. Mast cells,
5. Fibroblasts,
6. Synovial cells,
7. Keratinocytes,
8. Endothelial cells,
9. Stromal cells,
10. Mesangial cells,
11. Glial cell neurons,
12. Chondrocytes and osteoblasts,
13. Smooth muscle cells,
14. Adipocytes, and
15. Other cells including tumor cells

The synthesis of IL-6 is strictly regulated by transcriptional and post-transcriptional mechanisms **(Tanaka et al. 2014)**. Transcriptional factors such as nuclear factor kappa B (NF-kB), nuclear factor IL-6 (NF-IL6), activation protein-1, and interferon regulatory factor-1 activate the IL-6 gene.

The activation of receptors, such as glucocorticoid, estrogen, and aryl hydrocarbon, represses the IL-6 gene expression.

IL-6 stimulates hepatocytes to produce acute-phase proteins, such as C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen, hepcidin, and a1-antichymotrypsin, and it reduces the production of fibronectin, albumin, and transferrin.[23]This increase in the levels of acute-phase proteins produces an emergency stress signal, which, contributes to host defense.

Biological role

The Biologic activities of IL-6 is explained in Fig.1. It also has redundancy property (with those of other members of the IL-6 family of cytokines).

When tissue homeostasis is restored, the synthesis of IL-6 ceases. However, the dysregulated continuous production of IL-6 by distinct cell populations plays a pathologic role in various diseases.

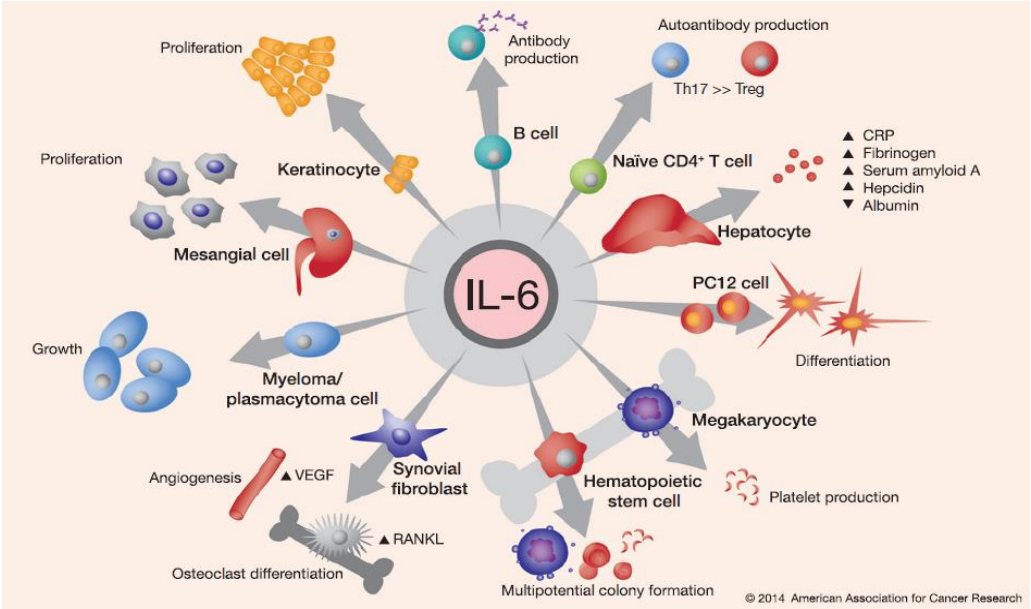


Figure 1. Pleiotropic activities of IL-6(Courtesy: The Biology and Medical Implications of Interleukin-6.Toshio Tanaka and Tadamitsu Kishimoto. Masters of immunology 2014.)

The immediate and transient expression of IL-6 contributes to host defence. When the source of stress is removed from the host, IL-6–mediated activation of the signal transduction cascade is ended by negative regulatory systems, such as ligand-induced internalization and degradation of gp130, and the recruitment of SOCS (Suppressors of cytokine signalling)[24], resulting in the normalization of serum levels of acute-phase proteins such as CRP and SAA (Serum amyloid A). At the same time, IL-6 synthesis ceases. But the dysregulated, persistent IL-6 production, through mostly unknown mechanisms (one of which may be due to an imbalance between Arid5a and Regnase-1) continues. This leads to the development of various diseases. The first association of IL-6 with disease development was demonstrated in a case of cardiac myxoma, in which the fluid obtained from the myxoma tissue of a patient contained a large quantity of IL-6[25].

Kishimoto et al 2005 stated that Dysregulated IL-6 production also occurs in synovial fluids of rheumatoid arthritis, swollen lymph nodes of Castleman disease, myeloma cells, peripheral blood cells etc. and in many tumor cells.[26]

Periodontitis is considered a local inflammatory/infectious process with systemic response, manifested by an increase of CRP, IL-6, and possibly hepcidin. Hepcidin is involved in iron homeostasis. It is hypothesized that individuals with chronic periodontitis without any systemic diseases, present lower haemoglobin(due to increased hepcidin level) levels than those with periodontal health.

There have been various studies on IL-6 levels in saliva, serum and GCF in gingivitis and periodontitis patients.

In **2004, Ide et al** found that chronic periodontitis patients undergoing an episode of subgingival scaling showed a significant elevation in circulating TNF-α and IL-6.[27]

In 2010, Increased local (GCF and saliva) IL-6 were correlated with the presence of gingivitis **(Becerik et al,[28]; Johanssen et al,[29]).**

Increased local (GCF and saliva) and systemic (serum) IL-6 levels were shown in periodontitis patients compared with healthy subjects **(Geivelis et al, 1993;Costa et al, 2010).**

The levels of systemic IL-6 also seem to be affected by periodontal treatment, with an increase in association to the short-term inflammatory response to therapy **(D'Aiuto et al,2005[30]; Tonetti et al,2007[31])**

D'Aiuto et al, 2004[32]; Marcaccini et al, 2009[33] stated that long-term reductions in the level of IL-6 were observed, when a clinical improvement in the periodontal status was obtained**.**

In studies done by **Loos et al.,[34]Anila Prabhu et al.,[35]Ebersole et al.,[36] and Ebersole and Cappelli,[37]**it was found that the periodontitis patients had higher CRP and IL-6 levels when compared to the periodontally healthy subjects.

Sun et al in 2009, found that the Patients with aggressive periodontitis have significantly elevated levels of plasma C-reactive protein and interleukin-6.[38]

In 2009, Gani, in his study found that Periodontitis results in higher systemic levels of CRP and IL-6.These elevated inflammatory factors may increase inflammatory activity in atherosclerotic lesions and potentially increasing the risk for cardiovascular events.[39]

Nakajima et al. in the year 2010 reported that CRP and IL-6 were significantly higher in periodontitis patients compared to healthy controls.[40]

In a study **by Keles et al 2014,** it was found that IL-6 levels were higher in chronic periodontitis patients when compared with gingivitis patients.[41]

In a study by **Haro et al (2016),** it was found that, the number of teeth with deepened periodontal pockets was associated with elevated serum IL-6 levels among subjects with an unfavourable lipid composition.[42]

In a study by **Solomon et al 2016**, it was found that IL-6 levels were higher in patients with periodontitis and atherosclerosis and it improved following non-surgical periodontal therapy.[43]

Beck et al in 2017 in his study found that high gingival inflammation, tooth loss, severe tooth loss, and severe disease PPC(Periodontal Profile Class) components were significantly associated with diabetes, coronary heart disease (CHD), high-sensitivity C-reactive protein, and interleukin (IL)-6, while only severe disease was associated with stroke.[44]

Delange in 2018 found that, Severe periodontitis was significantly associated with increased levels of IL-6 compared with those with none or mild periodontitis.[45]

HEPCIDIN AND ANEMIA OF INFLAMMATION

The anemia of chronic disease refers to the compromised production of erythrocytes associated with chronic inflammatory states, including cancer, chronic infection, or autoimmune diseases. Anaemia can also occur in the setting of severe, acute inflammation, such as critical illness, or in cases of milder but persistent inflammatory signals that occur in obesity, aging, and kidney failure. For these reasons, the name "anaemia of inflammation" may be more suitable than anaemia of chronic disease. It is considered the second most common form of anaemia worldwide.

Hepcidin is a potent regulator of iron homeostasis and is a possible mediator of the anemia of inflammation[46]. Hepcidin mRNA is induced in hepatocytes in response to inflammation[47]. The cytokines interleukin-1b (IL-1b) and interleukin-6 (IL-6)[48] have been demonstrated to be primarily responsible for hepcidin induction.

Serum and plasma hepcidin levels are elevated in conditions such as chronic kidney disease, inflammation, and multiple myeloma[49]. Hence, Hepcidin can be used as a therapeutic target for the treatment of AI (ANAEMIA OF INFLAMMATION) [50,51,52,53]. Mice[54,55] and humans[56] that produce elevated levels of hepcidin develop anemia that shares some features of Anaemia of inflammation (normocytic, normochromic type)[57].

Anemia of inflammation is associated with IL-6 in patients with rheumatoid arthritis[58], systemic lupus erythematosus[59], and the geriatric syndrome of frailty[60]. IL-6 not only induces hepcidin in these disease states but studies have proven that it also has direct oppressive effects on erythroid development in cultures[61,62].

As a result, the anaemia in these disease state is found to be multifactorial, resulting from hepcidin-mediated iron restriction as well as from direct effects of IL-6, on erythroid progenitor cells.

Pathogenesis of anemia of inflammation

Anaemia of inflammation is multifactorial, and the precise pathology is yet to be defined[63,64]. Erythrocyte survival is shortened due to macrophage activation by inflammatory cytokines and hemolysis. Erythropoiesis is impaired by inflammatory cytokines by inhibition of the production and function of erythropoietin, and by direct inhibition of erythroid progenitor cell proliferation and differentiation. Inflammatory

cytokines also induce the production of iron regulatory hormone hepcidin. It suppresses the iron exporter ferroportin to restrict the supply of iron for erythropoiesis.

HEPCIDIN

Hepcidin[65] is a peptide discovered in **2001 by park et al**. It is the key mediator of anaemia of inflammation.[66,67] It is a conserved 25–amino acid peptide produced in the liver and detectable in blood and urine.[68] Mice lacking hepcidin mRNA developed iron overload in the liver and pancreas, whereas iron scarcity in the macrophage-rich spleen.[69] Transgenic mice with overproduction of hepcidin died at birth of severe iron deficiency.[70] In these studies it was suggested that hepcidin inhibits iron absorption in the small intestine, the release of recycled iron from macrophages, and transport of iron across the placenta. In human studies, the patients with large hepatic adenomas and iron-refractory anaemia (which otherwise cannot be explained), overexpressed hepcidin mRNA in their tumours.

HEPCIDIN PRODUCTION:

The hormone hepcidin is produced in a two-step process consisting of conversion of an 84-amino-acid–long peptide, preprohepcidin by N-terminal cleavage of a 24-amino-acid signal peptide to give rise to prohepcidin. It is followed by a second cleavage of a 35-amino-acid peptide to produce the active compound 25-amino-acid hepcidin (hepcidin-25).

HEPCIDIN REGULATION:

Hepcidin transcription in the liver is controlled by a multifaceted interchange of signals between inflammation, iron status, and erythropoietic drive[71,72] which is explained in Fig.2.

1) Circulating and tissue iron-upregulates hepcidin and limit further iron entry,
2) Erythropoietic drive-suppresses hepcidin and increases iron availability for erythrocyte production.
3) Hepcidin induction by inflammation is thought to have evolved by nature to sequester iron from pathogenic microorganisms.

Hepcidin regulation by inflammation

Inflammatory cytokines (particularly IL6) regulate hepcidin transcription through the JAK-STAT3 pathway. There is a primary role for IL-6 in hepcidin induction in many infections, which include, streptococcus pneumonia and influenza A and most extracellular pathogen-associated molecular patterns. IL-6 knockout mice showed a lessened/absent hepcidin induction to these stimuli[73]. IL-22 has a minor role in hepcidin induction by Lipopolysaccharides[74]. IL-1 can also regulate hepcidin, either by inducing IL-6 dependent or by IL6-independent mechanisms[75,76]. In a study it was shown that IL-1β stimulates hepcidin and induces hypoferremia in mice by an unproven alternate pathway. In which there is induction of SMAD1/5/8 signalling. This pathway was thought to be the mechanism for hepcidin induction by commensal intestinal bacteria (relevant to inflammatory bowel disease).[77]

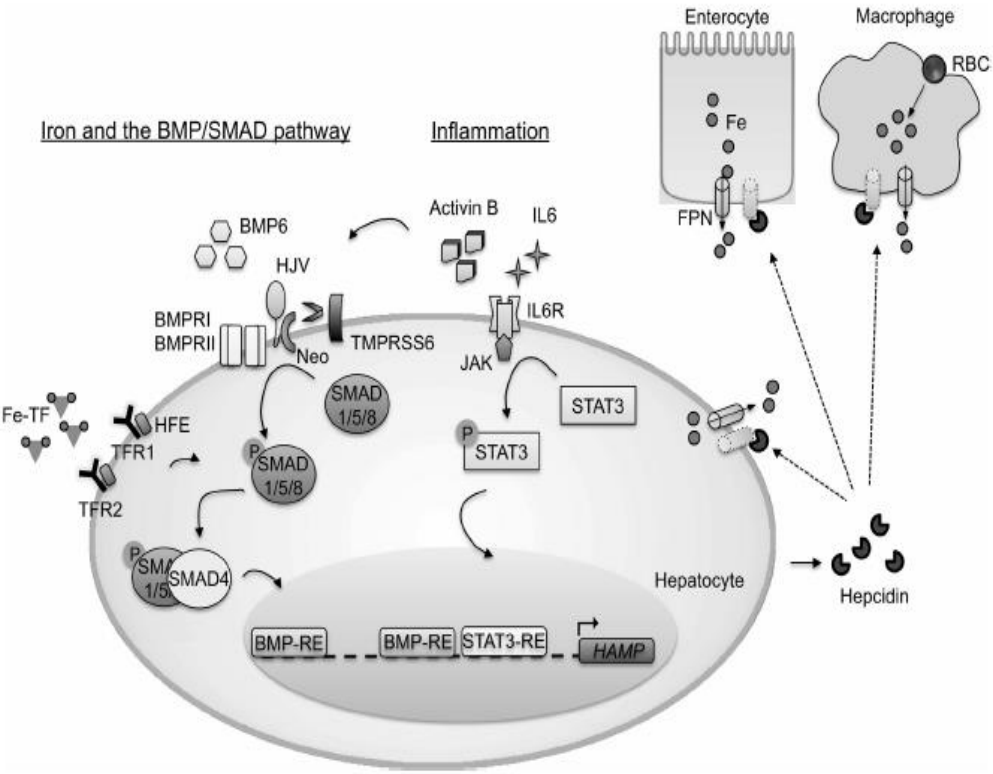


Figure2. Regulation of Hepcidin by various pathways (Courtesy: Hepcidin Regulation in the Anemia of Inflammation. Chia-Yu Wang and Jodie L. Babitt. CurrOpinHematol 2016)

Since, periodontitis is considered a local inflammatory/infectious process with a systemic response, it is speculated that patients with chronic periodontitis (not influenced by any systemic diseases) present lower hemoglobin levels than those with periodontal health owing to increased production of hepcidin **(Holtfreter et al, 2010).**[78]

In a study **by Shin Yu Lu et al in 2009,** a dramatic recovery from severe anaemia after resolution of severe periodontitis was observed.[79]

In 2010, a study by **Gokhale et al,** it was concluded that patients suffering from chronic periodontitis have a lower number of erythrocytes and hemoglobin compared to healthy controls.[80]

The findings of the study by **Anand et al in 2013**, suggested that patients with generalized aggressive periodontitis tend to have lower erythrocyte counts and lower hemoglobin levels compared with periodontally healthy controls.[81]

Patel in 2013, in his study concluded that like any other chronic condition, chronic periodontitis can lead to ACD (Anaemia of Chronic disease). It also provides evidence that non-surgical periodontal therapy can improve the anemic status of patients with chronic periodontitis.[82]

In 2013, Jenabian reported that, a correlation was observed between some hematological parameters associated with anemia and moderate chronic periodontitis.[83]

In 2013 Rao et al in a study found that there was statistically significant relation between oral hygiene, gingival inflammation, haemoglobin and total iron binding capacity in both the healthy and aggressive periodontitis group.[84]

In a study by **Hedge et al 2014**, Data analysis showed that patients with chronic periodontitis had lower values of hematocrit, number of erythrocytes, and hemoglobin and increased ESR level compared to healthy group. No remarkable differences in levels of MCH, MCHC and MCV were found between test and control group.[85]

Results of the study by **Nubesh Khan et al**, showed that patients suffering from chronic periodontitis had lower values of hemoglobin compared to healthy controls.[86]

Kolte et al in his study in 2014, concluded that periodontitis, like other chronic conditions, may lead to anemia as the number of erythrocytes is lower in affected patients.[87]

In 2016, Anumolu et al reported that, there was a decrease in Hb and erythrocyte counts and increase in white blood corpuscles counts in chronic generalized periodontitis when compared to healthy controls and chronic generalized gingivitis group. It was concluded that the treatment of periodontitis can lead to an improvement in hematocrit and other related blood parameters in chronic generalized periodontitis patients with anemia.[88]

In a study by **Mann et al in 2017,** remarkable hematological differences in EC (Erythrocytes), HGB (Haemoglobin concentration), MCV(Mean Corpuscular Volume) and MCH(Mean Corpuscular Haemoglobin) between healthy periodontium and chronic periodontitis subjects indicating mild anaemia were seen.[89]

In a study by **Parihar et al in 2018,** it was concluded that chronic periodontitis like any other chronic disease can cause anemia.[90]

STUDIES ON HEPCIDIN LEVELS

In 2011, Vilela et al found that Treatment of chronic periodontitis decreases serum prohepcidin levels in patients with chronic kidney disease.[91]

In 2015, Carvalho et al investigated the relationship between hepcidin and chronic periodontitis patients. It was the first investigation to estimate the association between periodontitis and the levels of hepcidin and haemoglobin in the blood.[92]

In the group of individuals with chronic periodontitis, both hematocrit values and those of serum iron were lower than in participants without periodontitis. The lowest

hematocrit value in this group can be attributed to less number of red blood cells in individuals with periodontal infection **(Kolte et al, 2014; Patel et al, 2014).**

The hypothesis generated in this study is that individuals without other diseases except periodontitis present lower levels of hepcidin and hemoglobin than those with periodontal health.

The rationale underlying this hypothesis is: In response to periodontal infection, cells from host defense system release immune-inflammatory mediators, such as IL-6, PGE2 etc., which stimulate the production of an acute phase protein called hepcidin **(Loos, 2005; Forner et al,2006; Yazdi et al, 2013).** Hepcidin plays a role in organic defense and homeostatic regulation of iron metabolism **(Ganz, 2003).**

This study showed that the level of serum hepcidin in individuals diagnosed with periodontitis was 65.6±14.3 ng ml^{-1}, and for those without such infection was 60.9±13.0 ng ml^{-1}.

In 2018, Guo et al, studied the Serum and salivary ferritin and Hepcidin levels in patients with chronic periodontitis and type 2 diabetes mellitus. The results were, Serum ferritin and hepcidin levels in the chronic periodontitis (CP) and CP with T2DM groups were higher than in the control group. Serum hepcidin and serum ferritin are linearly correlated. Serum hepcidin/ferritin values in the CP with T2DM group were significantly lower than those in the T2DM and control groups. Moreover, salivary ferritin levels in the CP and T2DM groups were higher than those in the control group. There was a positive correlation between salivary ferritin and serum ferritin. Hepcidin concentrations were relatively low in saliva.[93]

PERIODONTAL PHASE I THERAPY AND LEVELS OF SYSTEMIC PARAMETERS

Periodontitis is associated with elevated serum inflammatory markers **Pussinen et al 2007**. This suggests that periodontitis may have an impact on systemic health and the efforts to maintain or restore periodontal health may contribute to systemic health.

Studies have shown that biological markers of systemic conditions like atherosclerosis are more prevalent in periodontitis patients **Wick et al 2013.**

The currently available data on periodontal-systemic associations are rather heterogeneous **Linden et al 2013**, and the evidence that periodontal therapy has an impact on systemic health is limited and not yet established **D'Aiuto et al 2013**.

A few studies have tried to explain whether successful periodontal treatment can reduce the levels of serum markers and have yielded inhomogeneous results.

In a preliminary intervention study it was found that standard scaling and root planing (SRP) may change the levels of IL-6 and C-reactive protein **D'Aiuto et al 2004**. In a comparative study, SRP reduced levels of IL-6 and C-reactive protein, **D'Aiuto et al 2005**.

In another study by **Teles et al in 2012** it was found that periodontal therapy improved clinical and microbiological parameters but did not influence the levels of serum biomarkers.

Agarwal et al in 2009 in a study found that, correction of periodontal inflammation resulted in a significant increase in hemoglobin levels and erythrocyte counts. The erythrocyte sedimentation rate showed a reduction in periodontal inflammation. There was a significant, but much lesser, improvement in MCV, MCH and MCHC values.

These results showed that treatment of periodontitis leads to an improvement in hematocrit and other related blood parameters in chronic generalized periodontitis patients with anemia.[94]

Behle et al in 2009 in a study found that, Periodontal therapy resulted in an overall reduction of systemic inflammation, but the responses were inconsistent across subjects.[95]

Offenbacher et al. in 2009 reported that there was no significant difference in serum hsCRP at 6 months between patients receiving scaling and root planing and patients receiving community care[96].

In 2009 Marcaccini et al in a study found that, Circulating Interleukin-6 and High-Sensitivity C-Reactive Protein decrease after Periodontal Therapy in otherwise Healthy Subjects.[97]

Marcaccini et al in 2009 in another study showed that MMP-3, MMP-8, and MMP-9 concentrations were higher in periodontitis patients compared with healthy controls. In addition, non-surgical periodontal therapy significantly decreased plasma MMP-8 and MMP-9 concentrations by 35% and 39% compared to baseline; both results were statistically significant. The results suggested that plasma MMP-3, MMP-8, and MMP-9 may be useful biomarkers for the diagnosis of diseased individuals.[98]

In a pilot intervention study, **Duarte et al. in 2010** evaluated the serum concentration of several cytokines in patients with generalized chronic periodontitis, generalized aggressive periodontitis and healthy controls, and the effect of periodontal therapy on these cytokines 6 months after treatment. They found that TNF-alpha and IL-17 concentrations were higher in aggressive periodontitis patients compared to healthy

controls or chronic periodontitis, while there was no difference in other cytokines. In addition, nonsurgical periodontal therapy significantly decreased plasma TNF-alpha and IL-17 compared to baseline; both results were statistically significant; however, TNF-alpha levels remained elevated compared to healthy controls. The results suggested that serum TNF-alpha and IL-17 may be useful biomarkers for the differential diagnosis between aggressive and chronic periodontitis, while IL-17 may be useful for monitoring response to periodontal treatment in aggressive periodontitis.[99]

Pradeep et al in 2010, in a study found that Serum oncostatin M was significantly higher in patients with chronic periodontitis compared to healthy and gingivitis controls. Oncostatin M levels significantly decreased 2 months after nonsurgical periodontal therapy, and this decrease correlated with improvement in clinical parameters.[100]

Shimada et al in 2010 in a study on the Effect of Periodontal Treatment on Serum Leptin, Interleukin-6, and C-Reactive protein found that Periodontal treatment was effective in reducing serum leptin, IL-6, and CRP levels.[101]

In 2012, Malhotra et al in a study found that lower values of EC, Hb and HCT were seen in periodontitis group, which tends to improve after completion of periodontal therapy.[102]

Rastogi et al 2012 found that in patients with chronic periodontitis and known coronary artery disease, SRP reduced high-sensitivity C-reactive proteins (hsCRPs) and white blood cell counts.

Koromantzos, Makrilakis, Dereka, in their study in 2012, concluded that Effective non-surgical periodontal treatment of participants with type 2 DM (Diabetes Mellitus) and moderate to severe periodontal disease improved significantly the HbA1c levels.[103]

In 2014, Musalaiah et al in a study on Evaluation of nonsurgical periodontal therapy in chronic periodontitis patients with anemia by estimating hematological parameters and high sensitivity C-reactive protein levels showed that there was a significant increase in Hb levels, RBC count and PCV(Packed cell volume) from baseline to 6 months after nonsurgical periodontal therapy. There was significant decrease in levels of ESR and hs-CRP levels after nonsurgical periodontal therapy. There was a significant decrease in PPD, scores of PI and GI and significant increase in CAL gain.[104]

In 2016 Solomon et al in a study on the effect of non-surgical therapy on C reactive protein and IL-6 serum levels in patients with periodontal disease and atherosclerosis found that after the non-surgical periodontal therapy, the values of periodontal parameters and the levels of CRP and IL-6 had improved.

Jain et al in 2016, in a study on the effect of periodontal treatment on red blood cell parameters in patients with chronic periodontitis, found that periodontal therapy in patients with chronic periodontitis may lead to an increase in levels of red blood cells, hemoglobin concentration and packed cell volume together with decrease in values of ESR.[105]

Balli 2016 in a study on Chemerin and interleukin-6 levels in obese individuals following periodontal treatment found that greater values of chemerin and IL-6 were observed in obese individuals compared to their non-obese controls, and in individuals

with CP compared to their periodontal-healthy controls, which decreased following therapy.[106]

Yang 2017 in a study showed that Non-surgical periodontal treatment significantly reduced the serum ACPA (Anti-Citrullinated protein Antibodies) and TNF-α levels in CP patients, particularly in patients with generalized CP. There was a positive correlation between the number of extracted teeth and the reduction in the serum ACPA and IL-1β levels after non-surgical periodontal treatment.[107]

Haghoo JM 2018, in his study found that the amount of hemoglobin and red blood cells one month after treatment procedure noticeably increased in comparison to the base line. Also haematocrit showed improvement in laboratory results. While, LDL (Low density lipoprotein)/VLDL (Very low density lipoprotein) decreased in its value.[108]

The literature shows that periodontal disease is a local infection with a systemic inflammatory response. It has been shown that periodontitis is associated with various systemic complications, one of which is anaemia of chronic disease. But the exact mechanism for the development of anaemia of chronic disease due to periodontal disease is still unknown. Hepcidin is shown to be the key regulator of anaemia due to various chronic diseases. The relationship of hepcidin with periodontal disease is not established till now. Our study aims to,

- Establish the relationship between periodontal disease and serum Hepcidin and IL-6 levels.
- To evaluate the effectiveness of phase 1 periodontal therapy in reducing serum hepcidin and IL-6 levels.

MATERIALS AND METHODS

STUDY POPULATION:

The study population was selected from the outpatient section of the Department of Periodontology, Tamil Nadu Government Dental College and Hospital, Chennai.

STUDY DESIGN:

The study was of a case control type. The study participants were recruited prospectively in this study.

OPERATIONAL DEFINITIONS:

Chronic periodontitis- Chronic periodontitis has been defined as an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss and bone loss.

Aggressive periodontitis - Aggressive periodontitis is the disease of the periodontium occurring in an otherwise healthy individual which is characterised by a rapid loss of alveolar bone about more than one tooth of the permanent dentition.

Serum hepcidin - Hepcidin is a cationic peptide that is rich in cysteine. It is synthesized by the liver and excreted by the kidney and its main function is homeostatic regulation of iron metabolism.

Interleukin – 6 – it is a signalling protein/cytokine produced by several cells including macrophages, neutrophils, keratinocytes, and fibroblasts, in response to stimulus such as infection and trauma

Plaque Index (Sillness & Loe 1964) - An index for estimating the status of oral hygiene by measuring the microbial deposits which occurs adjacent to gingival margins

Bleeding Index (Ainamo & Bay 1975) - An assessment tool used to verify the presence of gum inflammation based on bleeding that occurs around gingival sulcus upon gentle probing with the standard periodontal probe.

Probing pocket Depth – Measurement of depth of gingival sulcus or periodontal pocket determined by measuring the distance from the gingival margin to the base of the sulcus or pocket with calibrated periodontal probe.

SAMPLING PROCEDURE:

The sampling was done by simple random sampling.

SAMPLE SIZE:

A total of 45 subjects were selected for the study.

Group I- 15 subjects with healthy periodontium. (Control group)

Group II- 15 subjects with chronic periodontitis (study group)

Group III- 15 subjects with aggressive periodontitis (study group)

ELIGIBILITY CRITERIA:

Inclusion criteria -

- Age 15– 55 years.
- Either sex.
- Minimum of 20 teeth should be present.

- Control group: group I- periodontally healthy individuals
- Study group:

a) Group II- patients with chronic periodontitis, with clinical attachment loss greater than or equal to 5mm, involving >30% of teeth present.

b) Group III- patients with aggressive periodontitis with attachment loss affecting at least 3 permanent teeth, other than the first molars and incisors

Exclusion criteria –

- Smokers.
- Infections(acute and chronic)
- Patients with systemic diseases, Organ transplantation and auto-immune diseases
- Medically compromised patients such as uncontrolled diabetes, hypertension, immunosuppression, bleeding disorders, cancer, stroke and severe osteoporosis.
- Patients with iron supplements to treat anaemia
- Patient who underwent periodontal treatment in past 6 months.
- Usage history of NSAIDs (non-steroidal anti-inflammatory drugs) and antibiotics within 3 months prior to the study
- Pregnancy and lactation
- Patients under bisphosphonates medication

ARMAMENTARIUM:

FOR CLINICAL EXAMINATION	FOR BLOOD SAMPLE COLLECTION	FOR PHASE 1 THERAPY
Mouth mirror	Sterile cotton	Mouth mirror
Curved explorer	Surgical spirit	Curved explorer
William's probe	Disposable 2 ml syringe with 22 gauge needle	William's probe
Dental tweezers		Hand-scalers
Sterile cotton rolls		Ultrasonic scaler unit (Manufacturer: woodpecker)
Sterilized disposable gloves		Curettes
Disposable head-caps		Kidney tray
Disposable facemasks		Sterile cotton rolls
Patient apron		Sterile gauze
		Disposable syringe with 24 gauge needle
		Local anaesthetic solution
		0.9% normal saline solution
		Patient apron

Selected patients in both study and control group were subjected to detailed case history, and clinical examination.

Clinical parameters assessed were:

- Plaque Index
- Bleeding on probing.
- Probing pocket depth
- Clinical attachment levels at four sites (mesial, facial , distal , palatal / lingual)

CLINICAL PARAMETER ASSESSMENT:

The following clinical parameters were evaluated for all the subjects:

1. Plaque index – ***Silness and Loe 1964***
2. Gingival bleeding index – ***Ainamo and Bay 1975***
3. Probing pocket depth in mm (PD) – ***Carranza 12th ed***
4. Clinical attachment level in mm (CAL) – ***Carranza 12th ed***

Plaque Index (Silness and Loe)

All teeth were examined at 4 sites each (disto-facial, facial, mesio-facial, lingual / palatal) and were scored as,

Scoring Criteria:

Score 0: No plaque in the gingival area.

Score 1: A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque is recognized only by running a probe across the tooth surface.

Score 2: Moderate accumulation of plaque within the gingival pocket and on the gingival margin and / or adjacent tooth surface that can be seen by the naked eye.

Score 3: Abundance of soft deposits within the gingival pocket and / or on the gingival margin and adjacent tooth surface.

Calculation:

Plaque index per tooth = Total score/4

Plaque index per individual = Total PI per tooth / Total number of teeth examined

Interpretation:

Score 0 – Excellent oral hygiene

0.1 to 0.9 – Good oral hygiene

1.0 to 1.9 – Fair oral hygiene

2.0 to 3.0 - Poor oral hygiene

Gingival Bleeding Index (Ainamo & Bay)

Starting distobuccally, the probe was gently inserted into the sulcus and run to the buccal and mesial surfaces of every tooth at an angle of about 45º. This was repeated for all the teeth present. Similarly probing was carried out at palatal/lingual sites. The total number of bleeding sites per tooth was thus recorded for every tooth except the third molar.

Scoring Criteria:

Positive score (1) - Presence of bleeding within 10 seconds

Negative score (0) - Absence of bleeding

$$\% \text{ of bleeding sites} = \frac{\text{Total number of positive score} \times 100}{\text{Total number of surfaces of all teeth}}$$

Probing Pocket Depth (PPD)

Probing Pocket Depths were measured from the gingival margin to the base of the pocket in mm using William's Periodontal Probe. The probe was walked within the gingival sulcus along the circumference of the tooth. Keeping the probe parallel to the

long axis of the selected tooth, six measurements were made per tooth (Mesiobuccal, Distobuccal, Midbuccal, Mesiolingual, Distolingual and Midlingual).

Clinical Attachment Level (CAL)

Clinical Attachment Level was measured from the Cemento – Enamel Junction (CEJ) to the base of the pocket using William‘s Periodontal Probe.

When the gingival margin was located on the anatomic crown, the level of the attachment was determined by subtracting from the probing depth, the distance from the gingival margin to the CEJ (Cemento enamel junction). If both were the same, the loss of attachment was calculated to be zero.

When the gingival margin coincided with the CEJ, the loss of attachment was calculated as equalling the probing depth.

When the gingival margin was located apical to the CEJ, the loss of attachment was greater than the probing depth and therefore the distance between the CEJ and the gingival margin were added to the PD.

Three measurements were made on the buccal aspect and three on the lingual aspect of each tooth – total of six sites per tooth (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual, and Distolingual).

RADIOGRAPHIC PARAMETERS:

- Radiographs were taken to assess the bone loss.

ROUTINE BLOOD INVESTIGATIONS DONE WERE:

- Haemoglobin percentage- Hb%
- Bleeding time(BT)

- Clotting time(CT)
- Total leukocyte count
- Differential leukocyte count
- Random blood sugar

PHASE-I PERIODONTAL THERAPY:

- After clinical examination and oral prophylaxis, phase-I periodontal therapy was done in four weeks.
- Routine blood investigations were done for all the subjects before doing treatment
- A test dose of 0.5ml of 2% lignocaine solution was administered to the all the subjects. Local anaesthetic solution was then injected at diseased sites and scaling and root planing was done. Oral hygiene instructions (OHI) were given.
- Then the patients in study groups were kept under maintenance phase and were recalled after three months. Again clinical examination and blood sample collection was done.

ESTIMATION OF SERUM HEPCIDIN AND INTERLEUKIN-6 LEVELS:

Blood Samples were collected for the control group at baseline and for the study groups at baseline before phase-I therapy and 3 months after phase-I therapy.

- 2ml of blood sample was drawn from the ante cubital vein, using a 2 ml syringe with 22 gauge needle, under aseptic conditions, and transferred to sterile test tubes coated with EDTA.

Blood samples were taken to IBMS (Institute of Biomedical Science), Chennai, for estimation of serum hepcidin and interleukin-6 levels.

HUMAN HEPCIDIN ELISA KIT (Bioassay Technology Laboratory)

This sandwich kit is for the accurate quantification of human Hepcidin (also known as HEPC) in serum, plasma, cell culture supernates, cell lysates, tissue homogenates.

ASSAY PRINCIPLE:

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). HEPC is added to the wells pre-coated with HEPC monoclonal antibody. After incubation a biotin-conjugated anti-human HEPC antibody is added and binds to human HEPC. After incubation, unbound biotin-conjugated anti-human HEPC antibody is washed away during washing step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human HEPC antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human HEPC. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

REAGENTS USED:

Standard Solution (4800pg/ml)-0.5ml

Pre-coated ELISA Plate-12 * 8 well strips

Standard Diluent-3ml

Streptavidin-HRP-6ml

Stop Solution-6ml

Substrate Solution A -6ml

Substrate Solution B -6ml

Wash Buffer concentrate (30x) -20ml

Biotin-Conjugate Anti-human HEPC Antibody -1ml

OTHER MATERIALS REQUIRED:

1. 37°C±0.5°C incubator

2. Microplate reader with 450 ± 10nm wavelength filter

3. Precision pipettes and disposable pipette tips

4. Clean tubes

5. Deionized or distilled water

6. Absorbent paper

SAMPLE COLLECTION:

Serum was allowed to clot for 10-20 minutes at room temperature. Centrifuged at 2000-3000 RPM for 20 minutes.

ASSAY PROCEDURE:

1. 50µl standard was added to standard well.

2. 40µl sample was added to sample wells and then 10µl anti-HEPC antibody was added to sample wells, then 50µl streptavidin-HRP to sample wells and standard

wells（Not blank control well. It was mixed well. The plate was covered with a sealer. Gently vibrated to mix well. It was Incubated 60 minutes at 37°C.

3. The sealer was removed and the plate was washed 5 times with wash buffer. Wells were soaked in wash buffer for 30 seconds to 1 minute for each wash. The plate was blotted onto paper towels or other absorbent material.

4. 50µl substrate solution A was added to each well and then 50µl substrate solution B was added to each well. The plate was covered with a new sealer for 10 minutes at 37°C at room temperature in the dark.

5. 50µl Stop Solution was added to each well, the blue color changes into yellow immediately.

6. The optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 30 min after adding the stop solution.

CALCULATION OF RESULTS:

A standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and a best fit curve through the points on the graph was drawn. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis. If the standard had been diluted, the concentration read from the standard curve was multiplied by the dilution factor.

HUMAN INTERLEUKIN-6 ELISA KIT (Bioassay Technology Laboratory)

This sandwich kit is for the accurate quantification of Human Interleukin 6（also known as IL-6）in serum, plasma, cell culture supernates, cell lysates, tissue homogenates.

This kit is a Enzyme-Linked Immunosorbent Assay (ELISA). IL-6 is added to the wells pre-coated with IL-6 monoclonal antibody. After incubation a biotin-conjugated anti-human IL-6 antibody is added and binds to human IL-6.After incubation unbound biotin-conjugated anti-human IL-6 antibody is washed away during a washing step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human IL-6 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human IL-6.The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

REAGENTS USED:

Standard Solution (640ng/L)-0.5ml

Pre-coated ELISA Plate-12 * 8 well strips

Standard Diluent-3ml

Streptavidin-HRP-6ml

Stop Solution-6ml

SubstrateSolution A-6ml

Substrate Solution B -6ml

Wash Buffer concentrate (30x)-20ml

Biotin-Conjugate Anti-human IL-6Antibody -1ml

OTHER MATERIALSREQUIRED:

1. 37°C±0.5°C incubator

2. Microplate reader with 450 ± 10nm wavelength filter

3. Precision pipettes and disposable pipette tips

4. Clean tubes

5. Deionized or distilled water

6. Absorbent paper

SAMPLE COLLECTION:

Serum was allowed to clot for 10-20 minutes at room temperature. Centrifuged at 2000-3000 RPM for 20 minutes.

ASSAY PROCEDURE:

1. 50µl standard was added to standard well.

2. 40µl sample was added to sample wells and then 10µl anti-IL-6 antibody was added to sample wells, then 50µl streptavidin-HRP to sample wells and standard wells（Not blank control well). The ingredients are mixed well. The plate was covered with a sealer. Incubated 60 minutes at 37°C.

3. The sealer was removed and the plate is washed 5 times with wash buffer. The wells were soaked in wash buffer for 30 seconds to 1 minute for each wash. The plate was blotted onto paper towels or other absorbent material.

4. 50µl substrate solution A was added to each well and then 50µl substrate solution B was added to each well. The plate was covered with a new sealer and incubated for 10 minutes at 37°Cat room temperature in the dark.

5. 50µl Stop Solution was added to each well, the blue color changes into yellow immediately.

6. The optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 30 min after adding the stop solution.

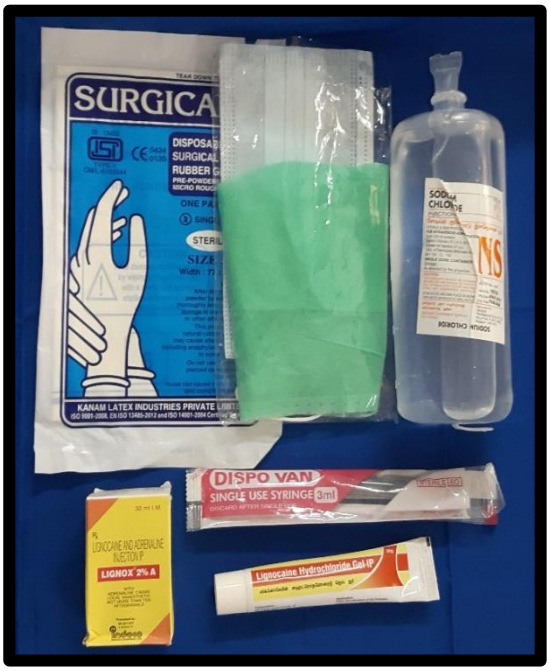
CALCULATION OF RESULTS:

A standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and a best fit curve through the points on the graph was drawn. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis. If the standard had been diluted, the concentration read from the standard curve was multiplied by the dilution factor.

PHOTOGRAPHS

Photograph 1: Armamentarium for examination of the patient

Photograph 2 & 3: Armamentarium for Phase I therapy and venous blood collection



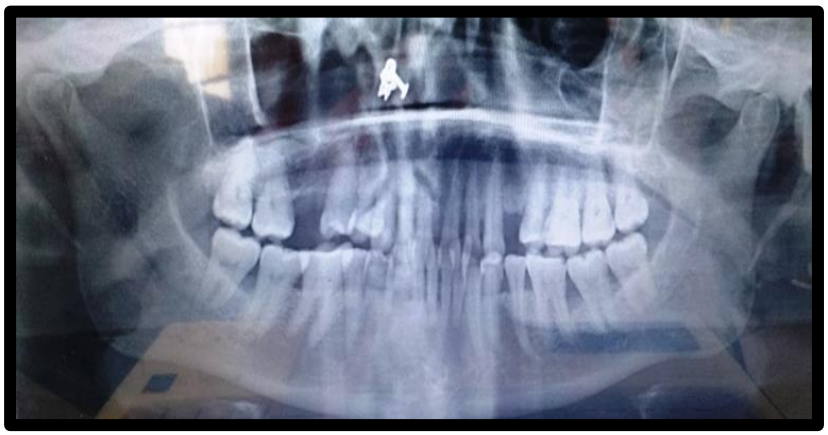
Photograph 4: Venous blood collection

Photograph 5: Group I- healthy subject at baseline

Photograph 6: Group II-Chronic periodontitis patient at baseline

Photograph 7: Group II-Chronic periodontitis patient at 3 months after Phase-I therapy

Photograph 8: Orthopantomograph of the chronic periodontitis patient

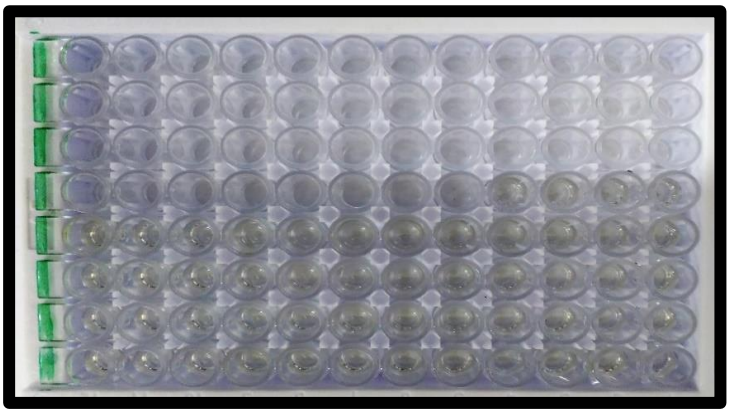


Photograph 9: Group III-Aggressive periodontitis patient at baseline

Photograph 10: Group III-Aggressive periodontitis patient at 3 months after phase-I therapy

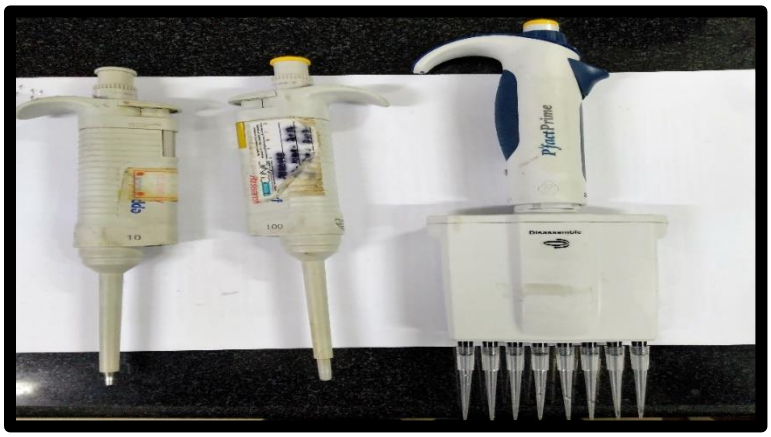
Photograph 11: Orthopantomograph of the Aggressive periodontitis patient

Photograph 12: ELISA plate with wells



Photograph 13: ELISA reader

Photograph 14: Pipettes



Photograph 15: Storage refrigerator for serum samples

Photograph 16: Cooling centrifuge

STATISTICAL ANALYSIS

In the present study, 45 subjects were selected, of which 15 were categorized as Group I which consisted of healthy subjects, 15 were categorized as Group II which consisted of patients with chronic periodontitis and another 15 were categorized as Group III which consisted of patients with aggressive periodontitis. The mean average of age in group I is 41.2±8.7, group II is 43.13±5.45 and group III is 25.8±5.6. The clinical parameters assessed were plaque index, gingival bleeding index, pocket probing depth, clinical attachment level and laboratory parameters assessed were serum IL-6,hepcidin level at baseline in group I, at baseline and 3 months after phase I therapy in group II and III.

The statistical analysis was done using the computer software program SPSS version 20.0 (Statistical Package for Social Science, Version 20).

Statistical Tests used were:

1. Paired t test
2. Unpaired t test
3. ANOVA
4. Post HOC analysis- Tukey's analysis

PAIRED t TEST

It is applied to paired data of independent observations from one sample only when each individual gives a pair of observations

UNPAIRED t TEST

This test is applied to unpaired data of independent observations made on individuals of two different or separate groups or samples drawn from two populations, to test if the difference between the means is real or it can be attributed to sampling variability.

ANALYSIS OF VARIANCE (ANOVA)

Many situations involve collecting data on three or more group of individuals, with the objective of determining whether any true differences in mean performance exist among the conditions under the study. This often happens in experimental situations where several different treatments may be under comparison. In the above situation, ANOVA is a way to test the equality of three or more means of more than two groups.

POST HOC ANALYSIS-TUKEY'S TEST

It consists of statistical analyses that were not specified before the data was seen. This typically creates a multiple testing problem because each potential analysis is effectively a statistical test. Multiple testing procedures are sometimes used to compensate, but that is often difficult or impossible to do precisely.

Tukey's test is used as a sequel to a significant analysis of variance test, to determine which of several groups are actually significantly different from one another. It has built-in protection against an increased risk of a type I error.

RESULTS

1. GINGIVAL BLEEDING INDEX

Intragroup comparison

Group I: The mean GBI at baseline was 11.32±2.08

Group II: The mean GBI at baseline was 81.36±6.29 and at 3 months was 23.97±3.86. The mean reduction in GBI from baseline to 3 months was statistically significant (p=0.000).

Group III: The mean GBI at baseline was 78.19±5.01 and at 3 months was 19.32±2.89. The mean reduction in GBI from baseline to 3 months was statistically significant (p=0.000).

Intergroup comparison

ANOVA comparing the mean baseline values among group I, group II and group III gave an F value of 1020.67 which was statistically significant(p=0.000).

POST HOC analysis for multiple comparisons of baseline GBI between group I and II, group I and III was statistically significant(p=0.000) and group II and III was not statistically significant(p=0.178)

Independent t tests to compare the mean 3 month values of PPD of group II and III gave a t value of 3.72 and was statistically significant(p=0.001)

2. PLAQUE INDEX

Intragroup comparison

Group I: The mean plaque index score at baseline was 0.71±0.12

Group II: The mean plaque index score at baseline was 2.66±0.21 and at 3 months was 0.85±0.21. The mean difference in plaque score from baseline to 3 month was statistically significant (p=0.000).

Group III: The mean plaque index score at baseline was 2.12±0.32 and at 3 months was 0.74±0.21. The mean reduction in plaque score from baseline to 3 months was statistically significant (p=0.000).

Intergroup comparison

ANOVA comparing the mean baseline values among group I, group II and group III gave an F value of 1821.9 which was statistically significant(p=0.000).

POST HOC analysis for multiple comparisons of baseline PI between group I and II, group I and III, group II and III was statistically significant(p=0.000)

Independent t tests to compare the mean 3 month values of PI of group II and III gave a t value of 1.43 and was not statistically significant(p=0.165)

3. PROBING POCKET DEPTH

Intragroup comparison

Group I: The mean PPD at baseline was 2.18±0.36

Group II: The mean PPD at baseline was 6.79 ±0.42 and at 3 months was 3.86±0.48. The mean reduction in PPD from baseline to 3 months was statistically significant (p=0.000).

Group III: The mean PPD at baseline was 7.18±0.42 and at 3 months was 4.69±0.48. The mean reduction in PPD from baseline to 3 months was statistically significant (p=0.000).

Intergroup comparison

ANOVA comparing the mean baseline values among group I, group II and group III gave an F value of 731.1 which was statistically significant(p=0.000).

POST HOC analysis for multiple comparisons of baseline PPD between group I and II, group I and III was statistically significant(p=0.000) and group II and III was statistically significant(p=0.029)

Independent t tests to compare the mean 3 month values of PPD of group II and III gave a t value of 4.77 and was statistically significant(p=0.000)

4. CLINICAL ATTACHMENT LEVEL

Intragroup comparison

Group I: The mean CAL at baseline was 0.000

Group II: The mean CAL at baseline was 6.79±0.45 and at 3 months was 3.89±0.48. The mean reduction in CAL from baseline to 3 months was statistically significant (p=0.000).

Group III: The mean CAL at baseline was 7.14±0.42 and at 3 months was 4.69±0.42. The mean reduction in CAL from baseline to 3 months was statistically significant (p=0.000).

Intergroup comparison

ANOVA comparing the mean baseline values among group I, group II and group III gave an F value of 1945.37 which was statistically significant(p=0.000).

POST HOC analysis for multiple comparisons of baseline CAL between group I and II, group I and III was statistically significant(p=0.000) and group II and III was statistically significant(p=0.023)

Independent t tests to compare the mean 3 month values of CAL of group II and III gave a t value of 4.88 and was statistically significant(p=0.000)

5. SERUM INTERLEUKIN-6 LEVEL

Intragroup comparison

Group I: The mean serum IL-6 level at baseline was 13.99±1.24

Group II: The mean serum IL-6 level at baseline was 18.65±2.84 and at 3 months was 13.87±1.21. The mean reduction in serum IL-6 level from baseline to 3 months was statistically significant (p=0.000).

Group III: The mean serum IL-6 level at baseline was 17.67±1.07 and at 3 months was 13.41±0.79. The mean reduction in IL-6 level from baseline to 3 months was statistically significant (p=0.000).

Intergroup comparison

ANOVA comparing the mean baseline values among group I, group II and group III gave an F value of 25.26 which was statistically significant(p=0.000).

POST HOC analysis for multiple comparisons of baseline serum IL-6 level between group I and II, group I and III was statistically significant(p=0.000) and group II and III was not statistically significant(p=0.341)

Independent t tests to compare the mean 3 month values of serum IL-6 levels of group II and III gave a t value of 1.24 and was not statistically significant(p=0.225)

6. SERUM HEPCIDIN LEVEL

Intragroup comparison

Group I: The mean serum Hepcidin level at baseline was 60.54±2.15

Group II: The mean serum Hepcidin level at baseline was 68.04±2.99 and at 3 months was 61.49±2.41. The mean reduction in serum Hepcidin level from baseline to 3 months was statistically significant (p=0.000).

Group III: The mean serum Hepcidin level at baseline was 65.79±1.79 and at 3 months was 60.42±1.99. The mean reduction in serum Hepcidin level from baseline to 3 months was statistically significant (p=0.000).

Intergroup comparison

ANOVA comparing the mean baseline values among group I, group II and group III gave an F value of 39.64 which was statistically significant(p=0.000).

POST HOC analysis for multiple comparisons of baseline serum Hepcidin levels between group I and II, group I and III was statistically significant(p=0.000) and group II and III wasstatistically significant(p=0.034)

Independent t tests to compare the mean 3 month values of serum Hepcidin levels of group II and III gave a t value of 1.33 and was not statistically significant(p=0.193)

Table 1: MASTER CHART 1-GROUP-I (CONTROL GROUP)-HEALTHY SUBJECTS

S.NO	AGE	SEX	GBI (%)	PPD (mm)	CAL (mm)	PI	SERUM IL-6 (pg ml^{-1})	SERUM HEPCIDIN (ng ml^{-1})
			BL	BL	BL	BL	BL	BL
1.	45	F	8.69	2.02	0	0.61	12.8	60.7
2.	45	M	9.02	2.52	0	0.53	15.2	61.2
3.	55	M	11.52	2.71	0	0.83	12.5	61.5
4.	47	M	8.78	2.63	0	0.79	14.7	54.4
5.	40	M	12.11	1.88	0	0.89	12.2	60.2
6.	43	M	9.92	2.17	0	0.58	14.5	60.5
7.	35	F	14.08	1.54	0	0.67	14.4	61.8
8.	42	F	8.52	1.9	0	0.59	13.7	64.2
9.	48	F	13.61	2.43	0	0.72	17.2	60.1
10.	34	M	10.32	2.13	0	0.68	14.1	60.8
11.	49	F	11.86	2.26	0	0.81	13.8	61.4
12.	50	F	12.54	2.39	0	0.86	14.5	58.3
13.	30	F	13.36	2.49	0	0.58	12.9	60.7
14.	24	M	14.76	1.74	0	0.76	13.7	59.8
15.	31	M	10.84	1.81	0	0.64	13.6	62.5

[GBI-Gingival Bleeding Index, PPD-Probing Pocket Depth, CAL-Clinical Attachment Level, PI-Plaque Index]

Table 2: MASTER CHART 2-GROUP-II (STUDY GROUP)-CHRONIC PERIODONTITIS PATIENTS AT BASELINE AND 3 MONTHS AFTER PHASE I THERAPY

S.NO	AGE	SEX	GBI (%)		PPD (mm)		CAL (mm)		PI		SERUM IL-6 (pg ml^{-1})		SERUM HEPCIDIN (ng ml^{-1})	
			BL	3M	BL	3M	BL	3M	BL	3M	BL	3M	BL	3M
1.	50	F	75.22	17.53	6.23	3.43	6.12	3.36	2.45	0.93	22.7	16.2	75.5	65.9
2.	40	M	90.56	24.53	5.97	3.65	6.07	3.73	2.59	0.85	21.8	12.9	70.1	65.4
3.	51	M	86.83	28.61	6.66	4.32	6.45	4.29	2.38	1.09	21.1	15.8	69.8	60.7
4.	46	M	82.43	21.73	6.87	4.26	6.76	4.19	2.76	1.12	16.7	13.3	65.4	60.3
5.	43	F	80.24	26.96	7.02	3.53	7.12	3.61	2.97	0.74	18.8	14.1	66.5	59.1
6.	34	F	90.23	18.77	7.54	2.99	7.43	2.84	2.53	0.58	16.4	12.8	65.8	58.5
7.	50	M	72.34	19.74	6.76	3.65	6.81	3.73	2.61	0.73	16.2	12.1	71.3	64.8
8.	44	F	80.37	19.44	7.21	4.21	7.34	4.27	2.28	0.59	16.8	13.2	65.4	60.2
9.	40	F	85.57	23.82	6.98	3.63	7.08	3.83	2.73	1.18	21.9	14.3	70.2	62.4
10.	41	M	89.21	27.63	6.59	3.63	6.63	3.69	2.55	0.97	24.2	14.5	66.3	61.9
11.	45	M	75.55	28.91	6.37	4.23	6.24	4.16	2.74	0.63	16.1	12.8	69.8	63.5
12.	49	F	70.98	27.45	7.31	4.74	7.35	4.76	2.89	0.83	16.8	12.5	68.1	60.4
13.	35	F	78.93	23.64	6.51	4.38	6.56	4.41	2.91	1.06	17.1	14.9	65.3	58.8
14.	37	M	83.29	22.76	6.81	3.36	6.65	3.43	2.76	0.57	16.6	14.4	65.4	60.1
15.	42	F	78.65	27.97	6.99	3.75	7.17	3.85	2.82	0.83	16.5	14.3	65.7	60.3

[GBI-Gingival Bleeding Index, PPD-Probing Pocket Depth, CAL-Clinical Attachment Level, PI-Plaque Index]

Table 3: MASTER CHART 3-GROUP-III (STUDY GROUP)-AGGRESSIVE PERIODONTITIS PATIENTS AT BASELINE AND 3 MONTHS AFTER PHASE-I THERAPY

S.NO	AGE	SEX	GBI (%)		PPD (mm)		CAL (mm)		PI		SERUM IL-6 (pg ml^{-1})		SERUM HEPCIDIN (ng ml^{-1})	
			BL	3M	BL	3M	BL	3M	BL	3M	BL	3M	BL	3M
1.	26	F	75.26	23.91	6.94	4.96	6.83	4.89	1.72	0.53	17.6	13.8	63.8	60.6
2.	22	F	72.16	21.83	7.97	5.02	7.99	5.17	1.83	0.64	18.9	13.4	64.7	61.2
3.	21	M	72.52	19.31	6.71	4.73	6.83	4.82	2.57	0.79	16.4	12.3	64.3	58.3
4.	37	M	83.63	17.34	7.08	4.91	7.02	4.85	2.31	0.84	16.8	13.8	66.8	57.9
5.	36	F	81.45	15.52	7.53	5.18	7.47	5.12	1.92	0.81	17.9	13.4	67.1	60.7
6.	25	F	80.29	15.94	7.92	5.21	7.88	5.16	1.79	1.03	20.1	14.9	64.8	58.6
7.	22	F	77.69	16.82	7.46	4.86	7.32	4.72	1.88	0.69	16.8	12.7	63.9	57.3
8.	18	F	87.51	23.65	6.85	4.41	6.64	4.35	2.39	0.51	16.7	12.6	68.2	63.5
9.	23	F	79.52	22.72	6.99	4.47	6.82	4.33	2.33	0.94	18.4	14.1	70.1	63.7
10.	26	M	81.41	20.71	6.53	3.94	6.61	4.04	2.46	0.82	17.5	12.6	65.3	59.9
11.	30	M	78.75	19.98	6.81	4.09	6.92	4.13	2.52	0.51	16.8	12.9	65.8	60.6
12.	19	M	70.17	19.54	7.27	5.15	7.15	5.09	2.41	0.66	16.5	13.4	64.4	58.6
13.	30	F	73.63	15.61	7.25	5.06	7.21	5.01	1.79	1.18	18.5	14.1	67.3	62.7
14.	24	M	83.96	16.73	6.93	3.68	7.02	3.97	1.84	0.55	18.7	12.7	65.9	61.5
15.	28	F	74.84	20.15	7.38	4.57	7.42	4.62	1.96	0.61	17.4	14.5	64.5	61.1

[GBI-Gingival Bleeding Index, PPD-Probing Pocket Depth, CAL-Clinical Attachment Level, PI-Plaque Index]

Table 4: DESCRIPTIVE STATISTICS OF GROUP-I

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
GROUP 1 AGE	15	24	55	41.20	2.245	8.695
GROUP 1 GBI	15	8.52	14.76	11.3287	.53891	2.08718
GROUP 1 PPD	15	1.54	2.71	2.1747	.09114	.35298
GROUP 1 CAL	15	.00	.00	.0000	.00000	.00000
GROUP 1 PI	15	.53	.89	.7027	.02972	.11511
GROUP 1 SERUM IL6	15	12.20	17.20	13.9867	.31891	1.23512
GROUP 1 SERUM HEPCIDIN	15	54.40	64.20	60.5400	.55470	2.14835

[GBI-Gingival Bleeding Index, PPD-Probing Pocket Depth, CAL-Clinical Attachment Level, PI-plaque Index]

Table 5: DESCRIPTIVE STATISTICS OF GROUP-II

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
GROUP 2 AGE	15	34	51	43.13	1.407	5.449
GROUP 2 GBI BL	15	70.98	90.56	81.3600	1.62349	6.28773
GROUP 2 GBI 3M	15	17.53	28.91	23.9660	1.00055	3.87513
GROUP 2 PPD BL	15	5.97	7.54	6.7880	.10787	.41779
GROUP 2 PPD 3M	15	2.99	4.74	3.8507	.12308	.47669
GROUP 2 CAL BL	15	6.07	7.43	6.7853	.11645	.45100
GROUP 2 CAL 3M	15	2.84	4.76	3.8767	.12463	.48268
GROUP 2 PI BL	15	2.28	2.97	2.6647	.05216	.20202
GROUP 2 PI 3M	15	.57	1.18	.8467	.05340	.20680
GROUP 2 SERUM IL6 BL	15	16.10	24.20	18.6467	.73406	2.84300
GROUP 2 SERUM IL6 3M	15	12.10	16.20	13.8733	.31114	1.20503
GROUP 2 SERUM HEPCIDIN BL	15	65.30	75.50	68.0400	.77366	2.99638
GROUP 2 SERUM HEPCIDIN 3M	15	58.50	65.90	61.4867	.62051	2.40323

[GBI-Gingival Bleeding Index, PPD-Probing Pocket Depth, CAL-Clinical Attachment Level, PI-plaque Index]

Table 6: DESCRIPTIVE STATISTICS OF GROUP-III

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
GROUP 3 AGE	15	18	37	25.80	1.448	5.609
GROUP 3 GBI BL	15	70.17	87.51	78.1860	1.29269	5.00656
GROUP 3 GBI 3M	15	15.52	23.91	19.3173	.74734	2.89444
GROUP 3 PPD BL	15	6.53	7.97	7.1747	.10868	.42093
GROUP 3 PPD 3M	15	3.68	5.21	4.6827	.12349	.47829
GROUP 3 CAL BL	15	6.61	7.99	7.1420	.10685	.41382
GROUP 3 CAL 3M	15	3.97	5.17	4.6847	.10881	.42141
GROUP 3 PI BL	15	1.72	2.57	2.1147	.08102	.31378
GROUP 3 PI 3M	15	.51	1.18	.7407	.05179	.20059
GROUP 3 SERUM IL6 BL	15	16.40	20.10	17.6667	.27649	1.07083
GROUP 3 SERUM IL6 3M	15	12.30	14.90	13.4133	.20092	.77815
GROUP 3 SERUM HEPCIDIN BL	15	63.80	70.10	65.7933	.46378	1.79621
GROUP 3 SERUM HEPCIDIN 3M	15	57.30	63.70	60.4133	.51315	1.98741

[GBI-Gingival Bleeding Index, PPD-Probing Pocket Depth, CAL-Clinical Attachment Level, PI-plaque Index]

Table 7: PAIRED t TEST TO COMPARE THE MEAN VALUES BEFORE AND AFTER TREATMENT IN GROUP II

Variables		Paired Differences					t	df	Sig.
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	GROUP 2 GBI BL - GROUP 2 GBI 3M	57.39400	7.27146	1.87748	53.36720	61.42080	30.570	14	.000*
Pair 2	GROUP 2 PPD BL - GROUP 2 PPD 3M	2.93733	.64095	.16549	2.58239	3.29228	17.749	14	.000*
Pair 3	GROUP 2 CAL BL - GROUP 2 CAL 3M	2.90867	.65531	.16920	2.54577	3.27156	17.191	14	.000*
Pair 4	GROUP 2 PI BL - GROUP 2 PI 3M	1.81800	.27439	.07085	1.66605	1.96995	25.661	14	.000*
Pair 5	GROUP 2 SERUM IL6 BL - GROUP 2 SERUM IL6 3M	4.77333	2.38910	.61686	3.45029	6.09637	7.738	14	.000*
Pair 6	GROUP 2 SERUM HEPCIDIN BL - GROUP 2 SERUM HEPCIDIN 3M	6.55333	1.57882	.40765	5.67901	7.42765	16.076	14	.000*

***The differences between the mean values of all the variables before and after treatment are statistically significant ($p<0.05$)**

[GBI-Gingival Bleeding Index, PPD-Probing Pocket Depth, CAL-Clinical Attachment Level, PI-plaque Index]

Table 8: PAIRED t TEST TO COMPARE THE MEAN VALUES BEFORE AND AFTER TREATMENT IN GROUP III

Variables		Paired Differences					T	df	Sig.
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	GROUP 3 GBI BL - GROUP 3 GBI 3M	58.86867	5.90988	1.52593	55.59588	62.14145	38.579	14	.000*
Pair 2	GROUP 3 PPD BL - GROUP 3 PPD 3M	2.49200	.36717	.09480	2.28867	2.69533	26.286	14	.000*
Pair 3	GROUP 3 CAL BL - GROUP 3 CAL 3M	2.45733	.34100	.08804	2.26850	2.64617	27.910	14	.000*
Pair 4	GROUP 3 PI BL - GROUP 3 PI 3M	1.37400	.39520	.10204	1.15515	1.59285	13.465	14	.000*
Pair 5	GROUP 3 SERUM IL6 BL - GROUP 3 SERUM IL6 3M	4.25333	.89192	.23029	3.75940	4.74726	18.469	14	.000*
Pair 6	GROUP 3 SERUM HEPCIDIN BL - GROUP 3 SERUM HEPCIDIN 3M	5.38000	1.49914	.38708	4.54980	6.21020	13.899	14	.000*

***The differences between the mean values of all the variables before and after treatment are statistically significant (p<0.05)**

[GBI-Gingival Bleeding Index, PPD-Probing Pocket Depth, CAL-Clinical Attachment Level, PI-plaque Index]

Table 9: ANOVA TO COMPARE THE MEAN BASELINE VALUES OF THE PARAMETERS AMONG GROUP I, GROUP II AND GROUP III

Variables		Sum of Squares	df	Mean Square	F	Sig.
GBI	Between Groups	46921.825	2	23460.912	1020.668	.000*
	Within Groups	965.405	42	22.986		
	Total	47887.230	44			
PPD	Between Groups	232.162	2	116.081	731.099	.000*
	Within Groups	6.669	42	.159		
	Total	238.830	44			
CAL	Between Groups	485.881	2	242.940	1945.370	.000*
	Within Groups	5.245	42	.125		
	Total	491.126	44			
PI	Between Groups	343.664	2	171.832	1821.860	.000*
	Within Groups	3.961	42	.094		
	Total	347.625	44			
SERUM IL6	Between Groups	181.092	2	90.546	25.257	.000*
	Within Groups	150.568	42	3.585		
	Total	331.660	44			
SERUM HEPCIDIN	Between Groups	444.475	2	222.238	39.638	.000*
	Within Groups	235.481	42	5.607		
	Total	679.956	44			

*The differences between the mean baseline values of all the variables among group 1, group 2 and group 3 are statistically significant ($p<0.05$)

Table 10: POST HOC ANALYSIS FOR MULTIPLE COMPARISONS OF BASELINE GBI

VARIABLE	GROUPS		MEAN DIFFERENCE	STD. ERROR	SIG.	95% CONFIDENCE INERVAL	
						LOWER	UPPER
BASELINE GBI	GROUP 1	GROUP 2	70.03133	1.75065	.000*	65.7781	74.2845
		GROUP 3	66.85733	1.75065	.000*	62.6041	71.1105
	GROUP 2	GROUP 1	70.03133	1.75065	.000*	65.7781	74.2845
		GROUP 3	3.17400	1.75065	.178	1.0792	7.4272
	GROUP 3	GRUOP 1	66.85733	1.75065	.000*	62.6041	71.1105
		GROUP 2	3.17400	1.75065	.178	1.0792	7.4272

***The comparisons of baseline GBI are statistically significant (p<0.05)**

[GBI-Gingival Bleeding Index]

Table 11: POST HOC ANALYSIS FOR MULTIPLE COMPARISONS OF BASELINE PPD

VARIABLE	GROUPS		MEAN DIFFERENCE	STD. ERROR	SIG.	95% CONFIDENCE INERVAL	
						LOWER	UPPER
BASELINE PPD	GROUP 1	GROUP 2	4.61333	.14550	.000*	4.2598	4.9668
		GROUP 3	5.00000	.14550	.000*	4.6465	5.3535
	GROUP 2	GROUP 1	4.61333	.14550	.000*	4.2598	4.9668
		GROUP 3	.38667	.14550	.029*	.0332	.7402
	GROUP 3	GRUOP 1	5.00000	.14550	.000*	4.6465	5.3535
		GROUP 2	.38667	.14550	.029*	.0332	.7402

***The comparisons of baseline PPD are statistically significant (p<0.05)**

[PPD-Probing Pocket Depth]

Table 12: POST HOC ANALYSIS FOR MULTIPLE COMPARISONS OF BASELINE CAL

VARIABLE	GROUPS		MEAN DIFFERENCE	STD. ERROR	SIG.	95% CONFIDENCE INERVAL	
						LOWER	UPPER
BASELINE CAL	GROUP 1	GROUP 2	6.78533	.12904	.000*	6.4718	7.0988
		GROUP 3	7.14200	.12904	.000*	6.8285	7.4555
	GROUP 2	GROUP 1	6.78533	.12904	.000*	6.4718	7.0988
		GROUP 3	.35667	.12904	.023*	.0432	.6702
	GROUP 3	GRUOP 1	7.14200	.12904	.000*	6.8285	7.4555
		GROUP 2	.35667	.12904	.023*	.0432	.6702

***The comparisons of baseline CAL are statistically significant (p<0.05)**

[CAL-Clinical Attachment Level]

Table 13: POST HOC ANALYSIS FOR MULTIPLE COMPARISONS OF BASELINE PI

VARIABLE	GROUPS		MEAN DIFFERENCE	STD. ERROR	SIG.	95% CONFIDENCE INERVAL	
						LOWER	UPPER
BASELINE PI	GROUP 1	GROUP 2	1.41200	.11214	.000*	1.1396	1.6844
		GROUP 3	6.43933	.11214	.000*	6.1669	6.7118
	GROUP 2	GROUP 1	1.41200	.11214	.000*	1.1396	1.6844
		GROUP 3	5.02733	.11214	.000*	4.7549	5.2998
	GROUP 3	GRUOP 1	6.43933	.11214	.000*	6.1669	6.7118
		GROUP 2	5.02733	.11214	.000*	4.7549	5.2998

***The comparisons of baseline PI are statistically significant (p<0.05)**

[PI-Plaque Index]

Table 14: POST HOC ANALYSIS FOR MULTIPLE COMPARISONS OF BASELINE SERUM IL-6

VARIABLE	GROUPS		MEAN DIFFERENCE	STD. ERROR	SIG.	95% CONFIDENCE INERVAL	
						LOWER	UPPER
BASELINE SERUM IL6	GROUP 1	GROUP 2	4.66000	.69137	.000*	2.9803	6.3397
		GROUP 3	3.68000	.69137	.000*	2.0003	5.3597
	GROUP 2	GROUP 1	4.66000	.69137	.000*	2.9803	6.3397
		GROUP 3	.98000	.69137	.341	.6997	2.6597
	GROUP 3	GRUOP 1	3.68000	.69137	.000*	2.0003	5.3597
		GROUP 2	.98000	.69137	.341	.6997	2.6597

***The comparisons of baseline serum IL-6 are statistically significant (p<0.05)**

Table 15: POST HOC ANALYSIS FOR MULTIPLE COMPARISONS OF BASELINE SERUM HEPCIDIN

VARIABLE	GROUPS		MEAN DIFFERENCE	STD. ERROR	SIG.	95% CONFIDENCE INERVAL	
						LOWER	UPPER
BASELINE SERUM HEPCIDIN	GROUP I	GROUP 2	7.50000	.86462	.000*	5.3994	9.6006
		GROUP 3	5.25333	.86462	.000*	3.1528	7.3539
	GROUP II	GROUP 1	7.50000	.86462	.000*	5.3994	9.6006
		GROUP 3	2.24667	.86462	.034*	.1461	4.3472
	GROUP 3	GRUOP 1	5.25333	.86462	.000*	3.1528	7.3539
		GROUP 2	2.24667	.86462	.034*	.1461	4.3472

***The comparisons of baseline serum Hepcidin are statistically significant (p<0.05)**

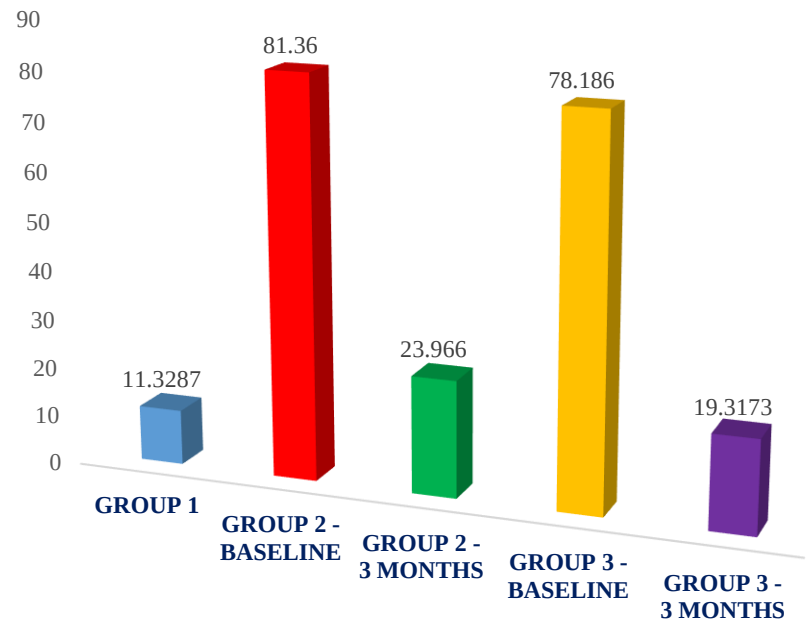
Table 16: INDEPENDENT t TESTS TO COMPARE THE MEAN 3 MONTHS VALUES OF THE PARAMETERS OF GROUP II AND GROUP III

Variables	Independent t-test						
	t	Df	Sig.	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower	Upper
GBI	3.722	28	.001*	4.64867	1.24885	2.09051	7.20682
PPD	4.772	28	.000*	.83200	.17436	1.18915	.47485
CAL	4.884	28	.000*	.80800	.16544	1.14689	.46911
PI	1.425	28	.165	.10600	.07439	.04638	.25838
SERUM IL6	1.242	28	.225	.46000	.37037	.29867	1.21867
SERUM HEPCIDIN	1.333	28	.193	1.07333	.80521	.57605	2.72272

***The differences between the mean 3 months values the variables of group II and group III are statistically significant (p<0.05)**

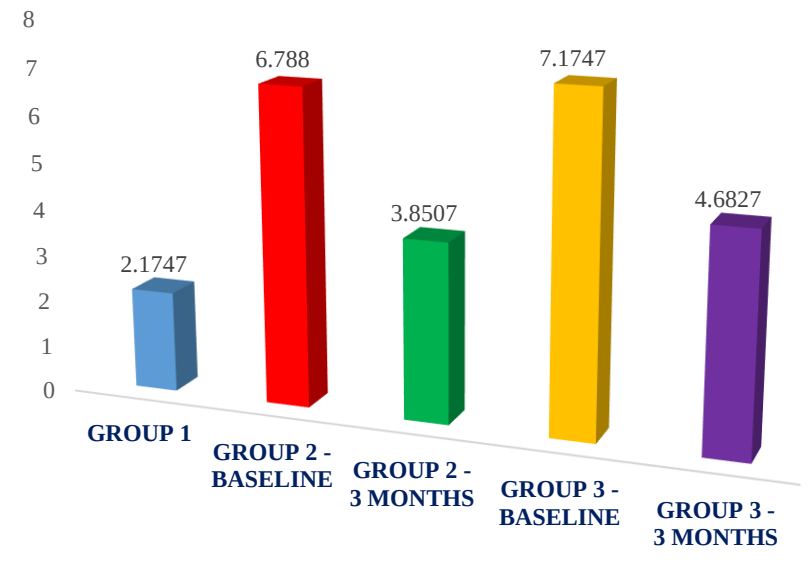
[GBI-Gingival Bleeding Index, PPD-Probing Pocket Depth, CAL-Clinical Attachment Level, PI-plaque Index]

Figure 3: COMPARISON OF MEAN GBI



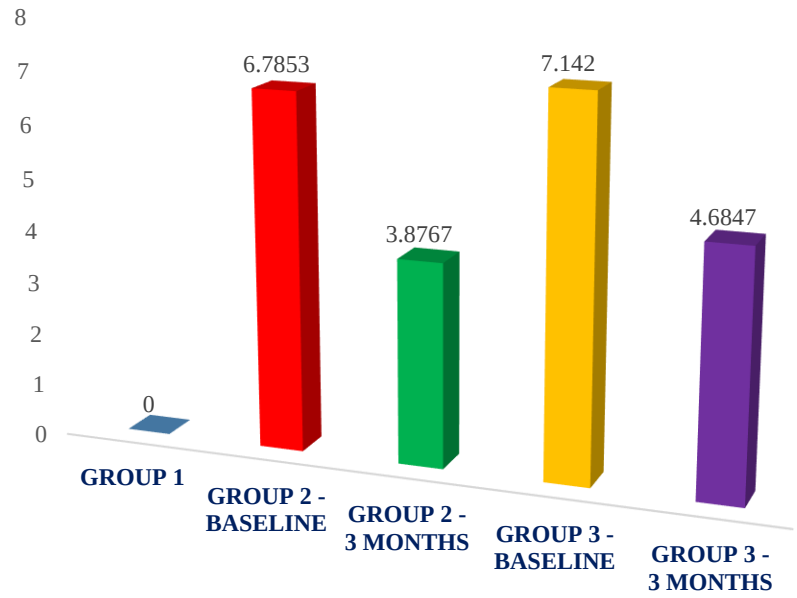
[GBI-Gingival Bleeding Index]

Figure 4: COMPARISON OF MEAN PPD



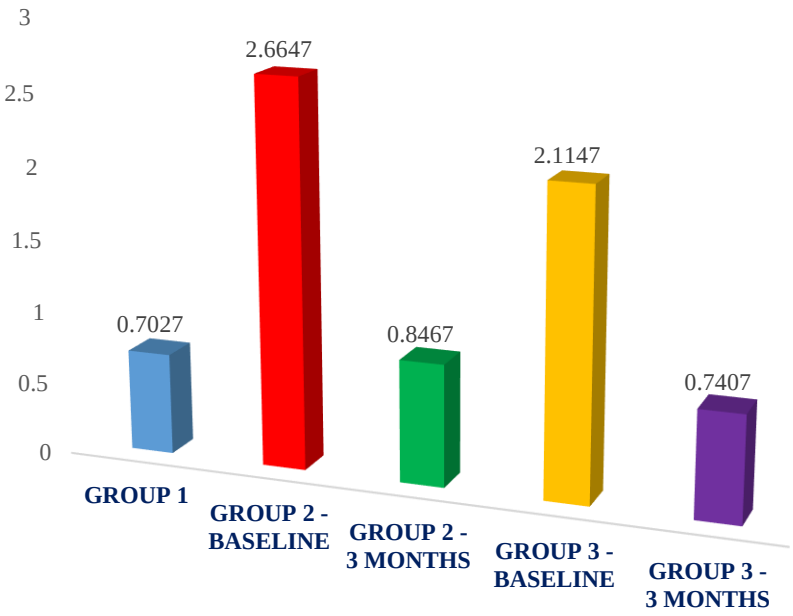
[PPD-Probing Pocket Depth]

Figure 5: COMPARISON OF MEAN CAL



[CAL-Clinical Attachment Level]

Figure 6: COMPARISON OF MEAN PI



[PI-Plaque Index]

Figure 7:COMPARISON OF MEAN SERUM IL-6

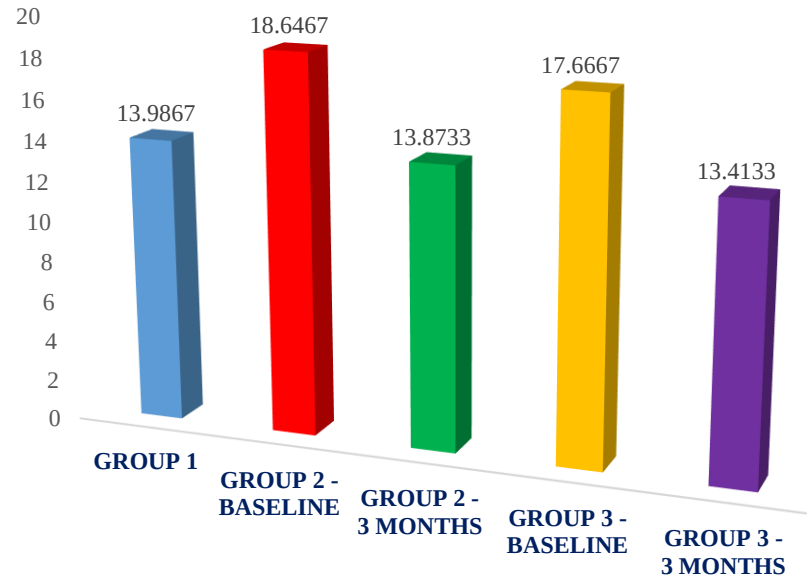
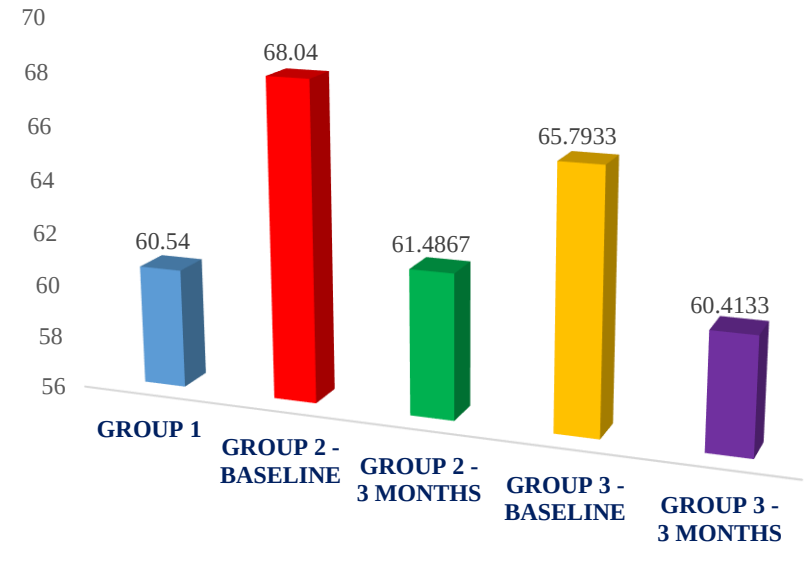


Figure 8: COMPARISON OF MEAN SERUM HEPCIDIN



DISCUSSION

Periodontal disease is a multifactorial chronic inflammatory disease that leads to a host response resulting in inflammatory breakdown of tooth-supporting osseous and soft tissues, in response to a microbial challenge. Although the presence of microbial organisms is required as the primary etiologic agents for disease initiation in periodontitis, it is inadequate for progression and increased severity of disease. The onset, progression, and severity of periodontal disease are related to the interaction between periodontal microorganisms and the host immune response. In response to bacterial endotoxins, production of acute phase proteins which cause tissue breakdown occurs as a part of host response.[109]

Substantial scientific data indicate that the localized infections characteristic of periodontitis can have a significant effect on the systemic health of humans and animals.[110] It has been proven that serum IL-6 levels are increased in periodontitis patients. This elevation of IL-6 can induce the hepatic expression of an acute phase protein called Hepcidin.

Hepcidin was discovered in **2001 by Park et al and** it is considered to play a key role in anaemia of inflammation. There are various studies showing that periodontal disease is associated with anaemia. It is hypothesized that elevation of serum Hepcidin and IL-6 can lead to anaemia in periodontitis patients.

Anaemia is associated with enhanced oxidative stress. The shortage of iron causes tissue hypoxia and affects the production of iron-containing antioxidant proteins, which tilts the balance to the oxidative side[111]. In an anaemic state, a relative decrease in oxygen perfusion into the tissues has been suggested to act as an altering factor in

the response of the periodontium to local irritation leading to periodontal destruction[112]. The literature thus proves the two-way relationship between the periodontal disease and anaemia.

Our study principally aims to,

- Establish the relationship between periodontal disease and serum Hepcidin and IL-6 levels.
- To evaluate the effectiveness of phase 1 periodontal therapy in reducing serum hepcidin and IL-6 levels.

In the present study, 45 subjects were enrolled, out of which 15 were healthy subjects (Group I), 15 were generalised chronic periodontitis patients (Group II) and 15 were generalised aggressive periodontitis(Group III) patients. Both sexes were included in the study.

Smokers, Patients with systemic diseases, Medically compromised patients such as uncontrolled diabetes, immunosuppression, bleeding disorders, cancer, stroke and severe osteoporosis, patients on iron replacement for treatment of anemia, patient who underwent periodontal treatment in the past 6 months, patients using NSAIDs and antibiotics within 3 months prior to the study, Pregnancy and lactation, Patients under bisphosphonates medication, Organ transplantation and auto-immune diseases were all excluded from the study.

Less consideration has been given to explore the role of chronic oral diseases on systemic health until recently. The hypothesis that periodontal infection, may be a risk factor for important medical outcomes, represents a paradigm shift in thinking about the association between the oral and systemic condition. The subgingival microbiota in patients with periodontitis provides a significant and persistent gram-

negative bacterial challenge to the host. Microbes and their products, have ready entree to the systemic circulation through the ulcerated/discontinuous sulcular epithelium/junctional epithelium found in periodontal diseases.[113] It has therefore been speculated that periodontitis results in a low grade systemic inflammation. Anaemia of inflammation is a cytokine mediated anaemia. It is frequently seen in clinical practice and is characterized by hypoferramia with adequate reticuloendotelial iron stores, normal to elevated ferritin concentrations and it is a regular complication of chronic inflammatory conditions[114,115]. **Hutteret al**[116] suggested that periodontitis has chronic and systemic effects and that periodontitis may tend towards anaemia. A tendency toward development of anaemia in patients with chronic periodontitis had been previously reported in various studies[117,118,119].

The expression of Interleukin-6 and Hepcidin during inflammation is thought to be the main reason for development of anaemia of chronic disease.

Vilela et al in 2011, found that treatment of chronic periodontitis decreases serum prohepcidin levels in patients with chronic kidney disease.

Carvalho et al in 2015 in his study found that both serum Interleukin-6 and Hepcidin levels were increased in chronic periodontitis patients. This was the first study to evaluate the Hepcidin levels in chronic periodontitis patients.

Lin-Na Guo in 2018 found that Serum ferritin and hepcidin levels in the Chronic periodontitis and Chronic periodontitis with Type2 diabetes mellitus groups were higher than in the control group.

Till now, there are no studies evaluating the Hepcidin levels in aggressive periodontitis patients and for evaluation of effectiveness of Phase 1 therapy in

reducing serum hepcidin levels in chronic and aggressive periodontitis patients. This provides the rationale for our study.

In our study, the baseline values of Interleukin-6 and Hepcidin levels in healthy subjects and chronic periodontitis patients were 13.99±1.24, 60.54±2.15 and 18.65±2.84, 68.04±2.99 respectively. The levels of IL-6 and Hepcidin were elevated in chronic periodontitis patients when compared with healthy subjects, which co-related with the results of the study from **Carvalho et al 2015.**

In aggressive periodontitis patients the values were, 17.67±1.07, 65.79±1.79 for Interleukin-6 and Hepcidin respectively which was found to be elevated when compared with healthy subjects.

ANOVA was used to compare the mean baseline values of all the parameters(GBI,PPD,PI,IL-6,Hepcidin) among groups I,II and III, which showed that the differences between the mean baseline values of all the parameters among group I,II and III were statistically significant($p<0.05$)

In Group II, the mean GBI at baseline was 81.36±6.29 and at 3 months was 23.97±3.86. The mean reduction in GBI from baseline to 3 months was statistically significant ($p=0.000$). The mean plaque index score at baseline was 2.66±0.21 and at 3 months was 0.85±0.21. The mean difference in plaque score from baseline to 3 month was statistically significant ($p=0.000$). The mean PPD at baseline was 6.79 ±0.42 and at 3 months was 3.86±0.48. The mean reduction in PPD from baseline to 3 months was statistically significant ($p=0.000$).

In group III, The mean GBI at baseline was 78.19±5.01 and at 3 months was 19.32±2.89. The mean reduction in GBI from baseline to 3 months was statistically significant ($p=0.000$). The mean plaque index score at baseline was 2.12±0.32 and at 3 months was 0.74±0.21. The mean reduction in plaque score from baseline to 3

months was statistically significant (p=0.000). The mean PPD at baseline was 7.18±0.42 and at 3 months was 4.69±0.48. The mean reduction in PPD from baseline to 3 months was statistically significant (p=0.000).

In both Group II and III, Gingival Bleeding Index, Plaque index and pocket probing depth reduced after 3 months following phase I therapy. This shows the efficiency of non-surgical therapy in improving periodontal health **(vilela et al 2011, Pradeep et al 2011).**

In group II, the mean serum Hepcidin level at baseline was 68.04±2.99 and at 3 months after phase I therapy was 61.49±2.41. The mean reduction in serum Hepcidin level from baseline to 3 months was statistically significant (p=0.000). This was similar to reports by **Guo et al 2018,** who reported that hepcidin levels reduced in chronic periodontitis patients after phase I therapy. The mean serum IL-6 level at baseline was 18.65±2.84 and at 3 months was 13.87±1.21. The mean reduction in serum IL-6 level from baseline to 3 months was statistically significant (p=0.000) which co-related with previous studies on IL-6 levels in chronic periodontitis patients after Phase I therapy by **Marcaccini AM 2009, Nakajima T 2010** etc.

In group III, the mean serum Hepcidin level at baseline was 65.79±1.79 and at 3 months was 60.42±1.99. The mean reduction in serum Hepcidin level from baseline to 3 months was statistically significant (p=0.000). The mean serum IL-6 level at baseline was 17.67±1.07 and at 3 months was 13.41±0.79. The mean reduction in IL-6 level from baseline to 3 months was statistically significant (p=0.000).

Ide et al 2003 reported that there was no statistically significant reduction in systemic inflammatory markers following periodontal therapy. However, **Behle et al 2009** reported that periodontal therapy resulted in overall reduction of various systemic inflammatory markers. In our study, we could positively correlate the levels of IL-6

and Hepcidin with periodontal disease status. The levels reduced following improvement in periodontal health in both group II and III. There was no statistically significant changes when comparing the mean 3 months values of IL-6 and Hepcidin of group II and III (p=0.225 and 0.193 respectively).

When compared with healthy subjects the levels of IL-6 and Hepcidin in aggressive periodontitis patients were elevated as in chronic periodontitis patients. The IL-6 and Hepcidin levels at baseline and at 3 months after Phase I therapy in Aggressive periodontitis patients were slightly less than in chronic periodontitis patients but the difference was not statistically significant. The response to periodontal phase I therapy in both the groups were similar.

This is the first study to evaluate the Hepcidin levels in aggressive periodontitis patients. Since IL-6 and Hepcidin are key regulators in development of anaemia of chronic disease. It is important to evaluate the relationship between periodontal disease and IL-6, Hepcidin levels.

It is a well-established fact that periodontal disease is associated with anaemia **(Anand et al 2014,Gokhale et al 2010)** and elevated serum IL-6 levels **(Geivelis et al, 1993; Costa et al, 2010; Shimada et al, 2010).** The systemic levels of IL-6 seem to be affected by periodontal treatment, with an increase associated to the short-term inflammatory response to therapy **(D'Aiuto et al, 2005; Tonetti et al, 2007)** and long-term reductions when a clinical improvement in the periodontal status is obtained **(D'Aiuto et al, 2004a; Marcaccini et al, 2009; Shimada et al, 2010).** But there are limited studies regarding Hepcidin levels in periodontitis patients and its response to periodontal phase I therapy. Our study investigated the serum IL-6 and hepcidin levels in chronic and aggressive periodontitis patients and compared it with healthy subjects. The changes in levels after Phase I therapy in both groups were also

evaluated. It was shown that periodontal phase I therapy improved the periodontal health and reduced IL-6 and hepcidin levels in both groups. This reduction in serum hepcidin and IL-6 levels will help in improving the systemic status of the patient and reduces the chances for development of anaemia due to periodontal disease. Future studies with a larger sample size are necessary to further establish the association between Hepcidin and periodontal disease.

SUMMARY AND CONCLUSION

Periodontal disease is a multifactorial inflammatory disease fundamentally initiated by chronic bacterial infection. In response to bacterial challenge the host produces various cytokines systemically. One of such cytokine is IL-6. Serum IL-6 level is found to be elevated in periodontitis patients. This elevation of Serum IL-6 levels can lead to increased production of an acute phase protein called Hepcidin by the liver. Hepcidin is considered the key regulator of anaemia of inflammation. It is speculated that increased levels of hepcidin and IL-6 in periodontal disease can cause anaemia. Anaemia causes oxidative stress and reduced oxygen perfusion in periodontal disease, leading to periodontal destruction. This proves the two-way relationship between anaemia and periodontal disease. All the above facts necessitates the need to explore the relationship between periodontal disease and anaemia.

The present study was conducted,

- To measure serum hepcidin and interleukin-6 levels in healthy subjects.
- To measure serum hepcidin and interleukin-6 levels in patients with chronic periodontitis.
- To measure serum hepcidin and interleukin-6 levels in patients with aggressive periodontitis.
- To compare serum hepcidin and interleukin-6 levels in individuals with healthy periodontium (control group) and chronic periodontitis(study group) and aggressive periodontitis(study group)
- To measure and compare serum hepcidin and interleukin-6 levels before and after phase I periodontal therapy in patients with chronic periodontitis.

- To measure and compare serum hepcidin and interleukin-6 levels before and after phase I periodontal therapy in patients with aggressive periodontitis.
- To compare the serum IL-6 and hepcidin levels between chronic and aggressive periodontitis patients.

A total of 45 patients were selected and divided into three groups of 15 each. Group I consisted of healthy subjects, Group II consisted of generalised chronic periodontitis patients, Group III consisted of generalised aggressive periodontitis patients. The clinical parameters and IL-6, Hepcidin levels were evaluated at baseline in group I, at baseline and 3 months after phase I therapy in group II and III. The values obtained were subjected to statistical analysis.

The following observations were made from our study:

- The IL-6 and Hepcidin levels were elevated in patients with chronic periodontitis when compared with healthy subjects.
- The IL-6 and Hepcidin levels were elevated in patients with aggressive periodontitis when compared with healthy subjects.
- The levels of IL-6 and hepcidin showed a considerable reduction 3 months after phase I therapy in both Groups.
- There was no statistically significant difference in IL-6 and Hepcidin levels at both baseline and 3 months after phase I therapy between chronic and aggressive periodontitis patients.
- All the clinical parameters showed a considerable improvement after phase I therapy in both Group II and III, and we can positively correlate the IL-6 and Hepcidin levels with the improvement in clinical parameters.

From the above outcomes, we could conclude that increased serum IL-6 and hepcidin levels are associated with both chronic and aggressive periodontitis patients. This elevation of serum hepcidin and IL-6 increases the risk for the development of anemia of chronic disease. In an anemic state, a relative decrease in oxygen perfusion into the tissues has been suggested to act as a modifying factor in the response of the periodontium to local irritation leading to periodontal destruction.

Following phase I therapy the levels of IL-6 and hepcidin levels were reduced in both chronic and aggressive periodontitis patients. The Phase-I therapy in the treatment plan of periodontal disease including both chronic and aggressive periodontitis patients proves to Improve the systemic status of the patient by preventing the development of anaemia due to periodontal disease.

In future, studies with a larger sample size may be employed,

1) To further establish the association between serum Hepcidin levels and periodontal status of the patient
2) To explore the association of serum IL-6 and Hepcidin levels in progression of periodontal disease.
3) To evaluate the efficiency of phase I therapy in reducing serum IL-6 and Hepcidin levels.

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Annexure-1

PARTICIPANT INFORMATION SHEET

TITLE OF THE STUDY:

"**A Comparative evaluation of Serum Hepcidin and interleukin-6 levels before and after phase I periodontal therapy in patients with chronic and aggressive periodontitis"**

NAME OF THE RESEARCH INSTITUTION:

Tamil Nadu Government Dental College and Hospital, Chennai.

PURPOSE OF THE STUDY:

The purpose of this study i**s** to **compare Serum hepcidin and interleukin-6 levels in patients with chronic and aggressive periodontitis before and after phase-I periodontal therapy.**

PROCEDURE:

- Case history will be taken, Intra Oral examination will be done, routine blood investigations will be done and X rays will be taken.
- About half-tea spoon(2ml) of blood will be drawn from your hand before starting the non-surgical treatment and it will be sent for estimation of serum hepcidin and interleukin-6 levels.
- Gums and deposits over the tooth will be examined.
- Cleaning of the tooth will be done under local anaesthesia.
- Then the blood sample(2ml-half tea spoon) will be collected again 3 months after the non-surgical treatment and it will be sent for estimation of serum hepcidin and interleukin-6 levels.

RISK OF PARTICIPATION:

- Radiation exposure during Intraoral periapical view radiographic procedure.
- Pain and discomfort due to treatment.
- Pain and swelling during blood sample collection and local anaesthetic injection. This will be informed to the patients and necessary precautions will be taken.

PROTECTION AND SAFETY MEASURES:

- Lead apron and thyroid collars will be used while taking radiographs.
- All instruments will be of standard quality and sterile.
- Analgesics will be prescribed after treatment if necessary.

BENEFITS OF PARTICIPATION:

Patient motivation and awareness regarding periodontal disease and its systemic effect. Scaling and root planing will be done for improvement of periodontal health.

CONFIDENTIALITY:

The identity of the patients participating in the research will be kept confidential throughout the study. In the event of any publication and presentation resulting from the research, no personally identifiable information will be shared.

PARTICIPANTS RIGHTS:

Taking part in the study is voluntary. You are free to decide whether to participate in the study or to withdraw at any time. Your decision will not result in any loss of benefits to which you are otherwise entitled.

COMPENSATION : Nil

_____ _____

Name of the participant **Signature of the participant**

For queries related to study:
Dr.S.Aiswarya,
PG student,
Department of Periodontics,
Tamil Nadu Government Dental College And Hospital,
Chennai-600003.

For queries related to rights of participant:
Dr.B.Saravanan,MDS
The Chairperson,
Institutional Ethical Committee,
Tamil Nadu Government Dental College and Hospital,
Chennai-600003.

Annexure 2

ஆராய்ச்சி பற்றிய தகவல் படிவம்

ஆராய்ச்சிமேற்கொள்பவர்: மருத்துவர்.ஸ்ரீ.ஐஸ்வர்யா
வழிநடத்துபவர்: மருத்தவர்.மஹேஷ்வரிஇராஜேந்திரன்

ஆராய்ச்சியின் தலைப்பு:

“நாட்பட்ட ஈறுஅழற்சிநோய் மற்றும் தீவிர ஈறுஅழற்சிநோய் உள்ளவர்களின் இரத்தத்தில் ஹப்சிடின் மற்றும் இன்டர்லூகின்-6 அளவை அறுவைசிகிச்சையில்லா ஈறுமருத்துவத்திற்கு முன்பும் பின்பும் ஒப்பிட்டு மதிப்பீடு செய்தல்"

செய்முறை:

கீழ்கண்ட ஆய்வுகள்/பரிசோதனைகள் உங்களுக்கு செய்யப்படும்:

- வாய் பரிசோதனை - உட்புறம், வெளிப்பறம்.
- நோயுற்ற பகுதியின் ஊடுகதிர்படம் எடுக்கப்படும்.
- வழக்கமான இரத்தப்பரிசோதனைசெய்யப்படும்.
- சிகிச்சைக்கு முன்பும் சிகிச்சைக்கு 3 மாதங்களுக்கு பின்பும் உங்கள் கையிலிருந்து பரிசோதனைக்காக 2மிலி (1/2 தேக்கரண்டி) அளவு இரத்தம் எடுக்கப்படும்.
- ஒவ்வாமை ஏற்படுகிறதா என்பதை தெரிந்துகொள்ள 0.5 மில்லி மரத்து போகும் மருந்து உங்களின் கையில் பரிசோதனைக்காக செலுத்தப்படும். பின்பு நோயுற்ற பகுதியில் இம்மருந்து செலுத்தப்படும்.
- அல்ட்ரா சோனிக் கருவி மற்றும் கைக்கருவிகள் பயன்படுத்தி பல் மற்றும் பல்லின் வேர் சுத்தம் செய்யப்படும். உப்புநீர் கொண்டு நோயுற்ற பகுதி சுத்தம் செய்யப்படும்.

பங்கேற்பதினால் வரக்கூடிய பக்கவிளைவுகள்:

- ஊடுகதிர் படம் எடுக்கும் பொழுது கதிர்வீச்சினால் பாதிப்பு ஏற்பட வாய்ப்பு உள்ளது.
- சிகிச்சைக்குப்பின் வலி ஏற்பட வாய்ப்பு உள்ளது.

- இரத்தம் எடுக்கும்பொழுதும் மரத்து போகும் ஊசி செலுத்தும் பொழுதும் வலி மற்றும் வீக்கம் வர வாய்ப்பு உள்ளது.

பக்க விளைவுகள் ஏற்படாமல் தடுக்க உரிய முறைகள் பின்பற்றப்படும்.

<u>பக்க விளைவுகள் ஏற்படாமல் தடுக்க பின்பற்றப்படும் முறைகள்:</u>

- ஊடுகதிர் படம் எடுக்கப்படும் பொழுது லெட்ஏப்ரன், தைராய்டு காலர் போன்ற பாதுகாப்பு உபகரணங்கள் பயன்படுத்தப்படும்.
- சிறந்த தரம் மற்றும் சுத்தமான கருவிகள் பயன்படுத்தப்படும்.
- தேவைப்பட்டால் சிகிக்சைக்குப்பின் வலி நிவாரணி மருந்துகள் வழங்கப்படும்.

பங்கேற்பதினால் விளையும் நன்மைகள்:

- ஈறு அழற்சி நோயினால் ஏற்படும் தீமைகளைப்பற்றி நோயாளிகளுக்கு விழிப்புணர்வு ஏற்படுத்தப்படும்.
- ஈறு அழற்சி நோய்க்கு சிகிச்சை அளிக்கப்படும்.

இரகசியகாப்பு:

உங்களை பற்றிய குறிப்புகள் பிறர் அறியாவண்ணம் ஆராய்ச்சி முடியும்வரை இரகசியமாக பாதுகாக்கப்படும். அதைவெளிப்படுத்தும் நேரங்களிள் எந்த தனிநபர் அடையாளங்களும் வெளிப்பட வாய்ப்பு கிடையாது.

தன்னார்வபங்கேற்பு:

இந்த ஆராய்ச்சியில் பங்கு பெறுவது தங்களின் தனிப்பட்ட முடிவு மற்றும் இந்த ஆராய்ச்சியிலிருந்து தாங்கள் எப்பொழுது வேண்டுமானாலும் விலகிக்கொள்ளலாம். தங்களின் இந்த திடீர் முடிவு தங்களுக்கோ அல்லது ஆராய்ச்சியாளருக்கோ எவ்வித பாதிப்பும் ஏற்படுத்தாது என்பதை தெரிவித்துக்கொள்கிறோம்.

_____ _____

நோயாளியின் பெயர் நோயாளியின் கையொப்பம்

ஆராய்ச்சி தொடர்புடைய
தொடர்புடைய தகவல்களுக்கு:

மருத்துவர்.ஸ்ரீ.ஐஸ்வர்யா,

முதுகலை மாணவர்,

தமிழ்நாடு அரசு பல் மருத்துவமனை
மற்றும் கல்லூரி, சென்னை-600003.

பங்கேற்பாளரின் உரிமை
தகவல்களுக்கு:

மருத்துவர்.பி.சரவணனன்,

தலைவர்,

நிறுவன நெறிமுறைகள் குழு,
தமிழ்நாடு அரசு பல் மருத்துவமனை

மற்றும் கல்லூரி, சென்னை-600003.

Annexure 3:

INFORMED CONSENT FORM

"A COMPARATIVE EVALUATION OF SERUM HEPCIDIN AND INTERLEUKIN-6 LEVELS BEFORE AND AFTER PHASE I PERIODONTAL THERAPY IN PATIENTS WITH CHRONIC AND AGGRESSIVE PERIODONTITIS"

"I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this study and understand that I have the right to withdraw from the study at any time without in any way it affecting my further medical care."

_____	_____	_____
Date	Name of the participant	Signature/Thumb impression Of the participant

[The literate witness selected by the participant must sign the informed consent form. The witness should not have any relationship with the research team; If the participant does not want to disclose his/her participation details to others, in view of respecting the wishes of the participant, he/she can be allowed to waive from the witness procedure. (This is applicable to literate participant ONLY). This should be documented by the study staff by getting signature from the prospective participant.]

"I have witnessed the accurate reading of the consent form to the potential participant and the individual has had opportunity to ask questions. I confirm that the individual has given consent freely."

_____	_____	_____
Date	Name of the witness	Signature of the witness

_____	_____	_____
Date	Name of the interviewer	Signature of the interviewer

Annexure 4

ஆராய்ச்சி ஒப்புதல்படிவம்

ஆராய்ச்சி தலைப்பு:

“நாட்பட்ட ஈறுஅழற்சிநோய் மற்றும் தீவிர ஈறுஅழற்சிநோய் உள்ளவர்களின் இரத்தத்தில் ஹப்சிடின் மற்றும் இன்டர்லூகின்-6 அளவை அறுவைசிகிச்சையில்லா ஈறுமருத்துவத்திற்கு முன்பும் பின்பும் ஒப்பிட்டு மதிப்பீடு செய்தல்"

பெயர்: வயது/பால்:

முகவரி: தொலைபேசி:

புறநோயாளிஎண்:

ஆராய்ச்சிசேர்க்கைஎண்:

நான் _____ வயது _____ என்னுடைய சுயநினைவுடனுன் மற்றும் முழு சுதந்திரத்திரத்துடனும் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக்கொள்ள ஒப்புதல் அளிக்கிறேன்.

கீழ்காணப்படும் நிபந்தனைகளுக்கு நான் சம்மதிக்கிறேன்:

நான் இந்த ஆராய்ச்சியின் நோக்கம் மற்றும் செய்முறைகள் பற்றி முழுமையாக தெரிவிக்கப்பட்டுள்ளேன்.

என் உடல்நலம் பாதிக்கப்பட்டாலோ அல்லது எதிர்பாராத வழகத்திற்குமாறான நோய்குறிகள் தென்பட்டாளோ அதற்கு சிகிச்சை பெற்று கொள்வதற்கும் முழு உரிமை உள்ளதாக அறிகிறேன்.

நான் எற்கனவே உட்கொண்ட மற்றம் உட்கொள்கின்ற மருந்துகளின் விபரங்களை ஆராய்சியாளரிடம் தெரிவித்துள்ளேன்.

என் மருத்துவகுறிப்பேடுகளை இந்தஆராய்ச்சியிள்
பயன்படுத்திக்கொள்ள சம்மதிக்கிறேன்.

இந்ந ஆராய்ச்சி மையமும் ஆராய்ச்சியாளரும் என்னுடைய விபரங்கள்
அனைத்தயும் இரகசியமாகவைப்பதாக அறிகிறேன்.

_____	_____	_____
நோயாளியின்பெயர்	கையொப்பம்	தேதி
_____	_____	_____
ஆராய்ச்சியாளரின்பெயர்	கையொப்பம்	தேதி

Annexure 5

DEPARTMENT OF PERIODONTICS

TAMILNADU GOVERNMENT DENTAL COLLEGE AND HOSPITAL

CHENNAI – 600 003

"**A COMPARATIVE EVALUATION OF SERUM HEPCIDIN AND INTERLEUKIN-6 LEVELS BEFORE AND AFTER PHASE I PERIODONTAL THERAPY IN PATIENTS WITH CHRONIC AND AGGRESSIVE PERIODONTITIS"**

PROFORMA

OP No: DATE: GROUP:

NAME: PATIENT ID No:

AGE/SEX:

ADDRESS:

MOBILE No:

OCCUPATION:

INCOME:

CHIEF COMPLAINT:

HISTORY OF PERSENTING ILLNESS:

PAST MEDICAL HISTORY:

PAST DENTAL HISTORY:

HISTORY OF HABITS:

INTRA ORAL EXAMINATION:

1) No. of teeth present:

2) Gingival Examination:

Colour:

Contour:

Consistency:

Texture:

Position:

Pigmentation:

3) Recession:

4) Mobility:

GINGIVAL BLEEDING INDEX

														28	38
														27	37
														26	36
														25	35
														24	34
														23	33
														22	32
														21	31
														11	41
														12	42
														13	43
														14	44
														15	45
														16	46
														17	47
														18	48
3	B												B	3	

CALCULATION

BASELINE

SCORE: **INFERENCE:**

3 MONTHS

SCORE: **INFERENCE:**

PROBING POCKET DEPTH AND CLINICAL ATTACHMENT LEVEL

3	CAL																
	PPD																
B	CAL																
	PPD																
3	CAL																
	PPD																
B	CAL																
	PPD																
		18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
		48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
B	PPD																
	CAL																
3	PPD																
	CAL																
B	PPD																
	CAL																
3	PPD																
	CAL																

PLAQUE INDEX – SILNESS AND LOE (1964)

		28	38		
		27	37		
		26	36		
		25	35		
		24	34		
		23	33		
		22	32		
		21	31		
		11	21		
		12	22		
		13	23		
		14	24		
		15	25		
		16	26		
		17	27		

		18	28		
3	B			B	3

INVESTIGATIONS

1) Blood investigation

Hemoglobin count

Total leucocyte count

Differential leucocyte count

Bleeding time

Clotting time

Random blood sugar

2) Serum Hepcidin and Interleukin-6 levels

SAMPLE	BASELINE	3 MONTHS
HEPCIDIN		
INTERLEUKIN-6		

DIAGNOSIS

PROGNOSIS

TREATMENT

PHASE-I:

S.NO	CALCULATIONS	BASELINE	3 MONTHS
1	Plaque index		
2	Gingival bleeding index		
3	Probing pocket depth		
4	Clinical attachment level		
5	Hepcidin		
6	Interleukin-6		

SIGNATURE OF PG STUDENT SIGNATURE OF GUIDE

DATE